Motor Unit Survival in Lifelong Runners Is Muscle Dependent

GEOFFREY A. POWER[1], BRIAN H. DALTON[1], DAVID G. BEHM[2], TIMOTHY J. DOHERTY[1,3], ANTHONY A. VANDERVOORT[1,4], and CHARLES L. RICE[1,5]

[1]Canadian Centre for Activity and Aging, School of Kinesiology, Faculty of Health Sciences, The University of Western Ontario, London, Ontario, CANADA; [2]School of Human Kinetics and Recreation, Memorial University of Newfoundland, St. John's, Newfoundland, CANADA; [3]Departments of Clinical Neurological Sciences and Rehabilitation Medicine, The University of Western Ontario, London, Ontario, CANADA; [4]School of Physical Therapy, Faculty of Health Sciences, The University of Western Ontario, London, Ontario, CANADA; and [5]Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, Ontario, CANADA

ABSTRACT

POWER, G. A., B. H. DALTON, D. G. BEHM, T. J. DOHERTY, A. A. VANDERVOORT, and C. L. RICE. Motor Unit Survival in Lifelong Runners Is Muscle Dependent. *Med. Sci. Sports Exerc.*, Vol. 44, No. 7, pp. 1235–1242, 2012. A contributing factor to the loss of muscle mass and strength with adult aging is the reduction in the number of functioning motor units (MUs). Recently, we reported that lifelong competitive runners (master runners = ~66 yr) had greater numbers of MUs in a leg muscle (tibialis anterior) than age-matched recreationally active controls. This suggested that long-term exposure to high levels of physical activity may limit the loss of MU numbers with adult aging. However, it is unknown if this finding is the result of long-term activation of the specifically exercised motoneuron pool (i.e., tibialis anterior) or an overall systemic neuroprotective effect of high levels of physical activity. **Purpose**: The purpose was to estimate the number of functioning MUs (MUNEs) in the biceps brachii (an upper body muscle not directly loaded by running) of nine young (27 ± 5 yr) and nine old (70 ± 5 yr) men and nine lifelong competitive master runners (67 ± 4 yr). **Methods**: Decomposition-enhanced spike-triggered averaging was used to measure surface and intramuscular EMG signals during elbow flexion at 10% of maximum voluntary isometric contraction. **Results**: Derived MUNEs were lower in the biceps brachii of runners (185 ± 69 MUs) and old men (133 ± 69 MUs) than the young (354 ± 113 MUs), but the old and master runners were similar. **Conclusions**: Although there were no significant differences in MUNE between both older groups in the biceps brachii muscle, with the number of subjects tested here, we cannot eliminate the possibility of some whole-body neuroprotective effect. However, when compared with the remote biceps muscle, a greater influence on age-related spinal motoneuron survival was found in a chronically activated MN pool specific to the exercised muscle. **Key Words:** AGING, PHYSICAL ACTIVITY, MUSCLE FUNCTION, MASTER ATHLETES, EMG

BASIC SCIENCES

Sarcopenia, associated with healthy adult aging and characterized by the loss of muscle mass, is preceded by the death of functioning motor units (MUs) (37) in addition to other intrinsic factors (11) leading to reductions in strength and power. However, high levels of long-term activity demonstrate favorable effects on the maintenance of MU numbers well into old age in both animal and human models (23,36). Thus, it remains unknown whether high levels of physical activity provide a systemic benefit to the neuromuscular system or if the effect is isolated to the motoneuron (MN) pool directly involved in the specific long-term activity.

Address for correspondence: Charles L. Rice, Ph.D., FACSM, School of Kinesiology, Faculty of Health Sciences, Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, Ontario, Canada N6G 1H1; E-mail: crice@uwo.ca.
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The loss of MUs occurs in a regressive manner, with the denervation of muscle preceding the loss of motor axons and eventual α-MN death (10,16). As a compensatory mechanism, healthy MUs (often Type I) can sprout new collateral axonal branches to reinnervate those muscle fibers orphaned (often Type II) after the death of their parent MN. Because this process does not recapture all denervated fibers, there is a gradual loss of muscle mass leaving the whole muscle composed of fewer and larger Type I MUs (15). The rate of this process may vary by muscle group (9,30), but eventually, some limit in the size of the remaining MUs is reached and the continued loss of MUs, and hence, sarcopenia may occur at a faster rate in very old age (30). The number of functioning MUs in a human muscle group or a MU number estimate (MUNE) can be determined electrophysiologically by dividing the compound muscle action potential (M wave), which represents the "maximal electrical size" of the muscle group, by a representative sample of surface-detected MU potentials (S-MUP), "electrical size" of an individual MU (see Bromberg (4) for review).

Age-related reductions of ~40% to 60% in functioning MU numbers have been reported for several human limb muscles, including the biceps brachii (6,13), extensor digitorum brevis

(14,33), tibialis anterior (30), and small intrinsic hand muscles (5,13,14). However, Dalton et al. (9) did not find a significant reduction in MUNE for the soleus, and because MU numbers are lower in very old adults (>90 yr) (40), the authors suggested that the loss of MUs is likely delayed in the highly active primarily slow-twitch (21) postural soleus muscle of healthy ambulatory adults. These findings provided indirect evidence of the possible role that habitual activity and perhaps fiber type may have on the rate of MU loss with healthy adult aging.

As demonstrated in rats (23) and, recently in humans (36), long-term physical activity can mitigate some of the MU loss associated with natural aging, although the "type" of activity may play an important role. In a rat model of resistance training, hindlimb overloading through synergist ablation induced hypertrophy but did not maintain MN numbers with adult aging (24). In contrast, moderate whole-body endurance exercise (30 min of swimming 3 $d \cdot wk^{-1}$) had a protective effect on MN numbers compared to age-matched controls (23). In addition, increased neuromuscular activity (through partial denervation of a spinal nerve root) in an ALS transgenic mouse model prevented the large (40%–70% loss) reduction in MN usually observed in this particular diseased mouse model of accelerated neuromuscular aging (16). It seems, based on the findings from the few available studies, that lifelong physical activity of moderate intensity may be more effective in preserving MU numbers than short bouts of resistance-type training.

The benefits of exercise on the maintenance of MNs may only provide a localized effect. For example, an investigation of caloric restriction and exercise in mice showed a whole-body maintenance of MNs in the calorie-restricted group, whereas only those MNs associated with the exercised limb were maintained in old age (38). Our recent study demonstrated that MUNE for the tibialis anterior muscle of lifelong master runners were greater than those from age-matched (~65 yr) healthy controls and were comparable to those from younger adults (36). Considering that lifelong running behavior involves primarily MNs of the lower extremity, our purpose was to determine whether the preservation of MNs associated with long-term physical activity was a systemic effect or localized to those MNs innervating the chronically active muscle groups. It was hypothesized that MUNEs in the less involved elbow flexors of master runners would not significantly differ from those obtained from age-matched controls and that both older groups would exhibit fewer functioning MUs than a sample of active young adults.

METHODS

Participants. Nine young men (27 ± 5 yr), nine old men (70 ± 5 yr), and nine master runners (one woman) (67 ± 4 yr) participated in this study. Young participants were recruited from the university population, and those who volunteered were considered to be recreationally active. The old men were also considered recreationally active for their age group. They attended a local seniors' exercise group not designed for systematic training but consisted of light walking and calisthenic activities three times per week for 60 min. All participants had no known neurological or cardiovascular diseases. The master runners were all training actively and competing for at least 30 yr. Recruitment was based on finishing times in a 10-mile (16-km) road race, and a detailed interview was conducted to identify highly trained lifelong runners (see Power et al. (36) for a table of self-reported training and performance data). The mean height and mass of the young and old men and master runners were 181.2 ± 7.0 cm and 80.8 ± 9.6 kg, 177.8 ± 6.7 cm and 90.1 ± 17.4 kg, and 174.2 ± 6.1 cm and 71.3 ± 10.5 kg, respectively. The study protocol was approved by the local university's ethics board and conformed to the Declaration of Helsinki. Informed oral and written consent were obtained before testing.

Experimental arrangement. Participants were asked to refrain from strenuous exercise 1 d before testing and not to consume caffeine on the day of testing. The left arm was secured to a custom isometric dynamometer with the arm at 110° of elbow flexion (terminal elbow extension being 180°) and the elbow supported in a padded cup with a 15°–20° shoulder abduction angle. The left shoulder was secured with a metal brace to eliminate extraneous body movements. The forearm was supinated fully, and the vertical aspect of the wrist was secured with a strap to a padded curved bar (11 × 5.2 cm) attached to the strain gauge (Model SST-700-100A; ASTechnology, Halliburton, Ontario, Canada). All testing was performed on the left arm (nondominant).

Surface EMG was collected from the biceps brachii, using self-adhering Ag/AgCl electrodes (1.5 × 1 cm; Marquette Medical Systems, Jupiter, FL). The skin was cleansed with alcohol before application of the electrodes. An active electrode was positioned over the motor point of the biceps brachii and a reference placed over the biceps tendon. A ground electrode was positioned between the stimulating electrode (positioned over the musculocutaneous nerve at the axilla) and the active surface electrode. Intramuscular EMG signals were recorded via a disposable concentric needle electrode with a recording surface of 0.03 mm^2 (Model N53153; Teca, Hawthorne, NY) inserted into the biceps brachii, 5–10 mm distal to the active surface electrode.

Experimental procedures. EMG data were acquired using decomposition enhanced spike triggered averaging software on a NeuroscanComperio system (Neurosoft, El Paso, TX). The surface and intramuscular EMG signals were band-pass-filtered at 5 Hz to 5 kHz and 10 Hz to 10 kHz, respectively. Data collection started with determining the maximum twitch force and M wave responses. The twitch was evoked using a bar electrode held firmly over the musculocutaneous nerve at the axilla. A computer-triggered stimulator (model DS7AH; Digitimer, Welwyn Garden City, Hertfordshire, UK) provided the electrical stimulation at a pulse width of 100 μs and 400 V. The current was increased progressively until a plateau in M wave amplitude was

TABLE 1. Neuromuscular properties of the biceps brachii.

Group (n = 9)	MVC (N·m)	Pt (N·m)	CD (ms)	VA (%)	Target RMS (% MVC-RMS)	Mean MUFR (Hz)
Young	348.2 ± 150.5	36.9 ± 10.8	157.3 ± 14.6	98.4 ± 2.2	6.0 ± 2.6	12.5 ± 2.0
Old	209.4 ± 56.0**	13.1 ± 3.9**	170.7 ± 16.8	94.9 ± 4.1	8.5 ± 1.6	11.3 ± 1.9
Master runners	205.1 ± 51.9*	17.5 ± 5.4*	187.8 ± 17.0*	96.4 ± 4.1	13.3 ± 6.5	13.1 ± 1.3

Values are mean ± SD.
Master runners had weaker evoked peak twitch force (Pt) and MVC, with longer contraction duration (CD) than young men (* $P < 0.05$), whereas the old had similar contractile speeds to the young but lower twitch and MVC force (** $P < 0.05$). Target RMS (%MVC-RMS), mean MU firing rate (MUFR), and voluntary activation (VA) were similar among groups.

reached. The active surface electrode was repositioned to minimize the visible rise time of the M wave negative peak ensuring the electrode was over the motor point. At this point, the current was increased 15% to ensure activation of all motor axons. Next, three MVCs were performed of 3- to 5-s duration with at least 2 min of rest between attempts. A twitch contraction was electrically evoked 1 s before MVC to determine twitch contractile properties. Participants were provided visual feedback of the force and encouraged verbally. Voluntary activation was assessed using the interpolated twitch technique (2). During the third MVC, peak root mean square (RMS) value of the raw surface EMG was calculated and called MVC-RMS.

After the three MVCs, participants were given 5 min of rest. Next, the concentric needle electrode was inserted into the short head of the biceps brachii, and participants were then asked to match a target line of 10% MVC for all subsequent isometric contractions. The investigator manipulated the concentric needle to minimize rise times of the negative peak amplitudes of the first two to three detected MU potentials (MUPs). Repositioning of the needle was completed by either adjusting the depth of insertion or sampling from a new area. Participants were then asked to slowly ramp their elbow flexion force to the target line within 1–2 s and hold the contraction steady for 30 s at the target line, during which time both the intramuscular and surface EMG were obtained simultaneously and stored for further analysis. Participants were given at least 1 min of rest between these contractions. The procedures were repeated until at least 20 suitable MUP trains and their respective S-MUP were sampled (3).

Data reduction and statistics. All offline analyses were completed by the same experienced operator using previously defined criteria (3). Decomposed EMG signals were reviewed offline to ensure their accuracy. To determine the acceptability of the needle-detected MUP trains and their corresponding S-MUPs, first, an acceptable MUP train required greater than 50 detected discharges that acted as triggers for spike-triggered averaging of the surface EMG signal. Then, the MU discharge pattern was inspected visually for a stable and physiological rate (i.e., coefficient of variation ≤30%). Lastly, the interdischarge interval histogram was examined to confirm that it followed a Gaussian distribution. When MUP trains did not meet these criteria, they were excluded from further analysis. Next, S-MUPs were inspected to identify a distinct waveform that was temporally linked to the needle potential (within 10 ms). The computer-generated negative peak onset and negative peak amplitude markers of the acceptable S-MUPs were inspected to ensure they were accurate. Any markers not correctly set were repositioned manually. A computer algorithm then aligned the negative onset markers for all accepted S-MUPs and created a mean S-MUP template based on their data point by data point average (12). Finally, a MUNE was derived by dividing the negative peak amplitude of the M wave by the negative peak amplitude of the mean S-MUP.

Force data were sampled at a rate of 1000 Hz and digitized using a 12-bit analog-to-digital converter (model 1401 Plus; Cambridge Electronic Design, Cambridge, UK). Spike2 software (Cambridge Electronic Design) was used during offline analysis to determine voluntary and evoked isometric forces and contraction duration (time-to-peak twitch force + half-relaxation time) of the evoked twitch.

All data were analyzed using SPSS v. 16 (Chicago, IL). A univariate ANOVA was performed to identify differences between groups for all EMG and force parameters. When a significant main effect was present, a Tukey HSD *post hoc* test was performed to identify where significant differences existed. A Mann–Whitney U test was used to test for statistical significance of voluntary activation between groups. Effect sizes (ES) were calculated using the η^2 method to explore the strength of apparent statistical effects, and 95% confidence intervals (95% CI) for the differences in means were calculated, as appropriate. A Pearson correlation coefficient (r) and a linear regression analysis (R^2) were performed to evaluate the relationship between age, M wave, and S-MUP for the biceps brachii. A second Pearson correlation coefficient (r) and a linear regression analysis (R^2) were performed to evaluate the relationship and shared variance between previously reported MUNE for the tibialis anterior of master runners (36) and the biceps brachii of those same individuals compared to young and old men. The level of significance was set at $P < 0.05$. A power calculation using G*Power (version 3.1.3) was performed to calculate the ES based on nine subjects to achieve a satisfactory power of $1 - \beta = 0.8$, which was then multiplied by Cohen d to determine the minimally detectable difference. All data are presented as means ± SD.

RESULTS

Strength, voluntary activation, and twitch properties. The young and old groups were of similar mass and stature, but the master runners were, on average, slightly lighter than the old men ($P = 0.01$). The master runners and

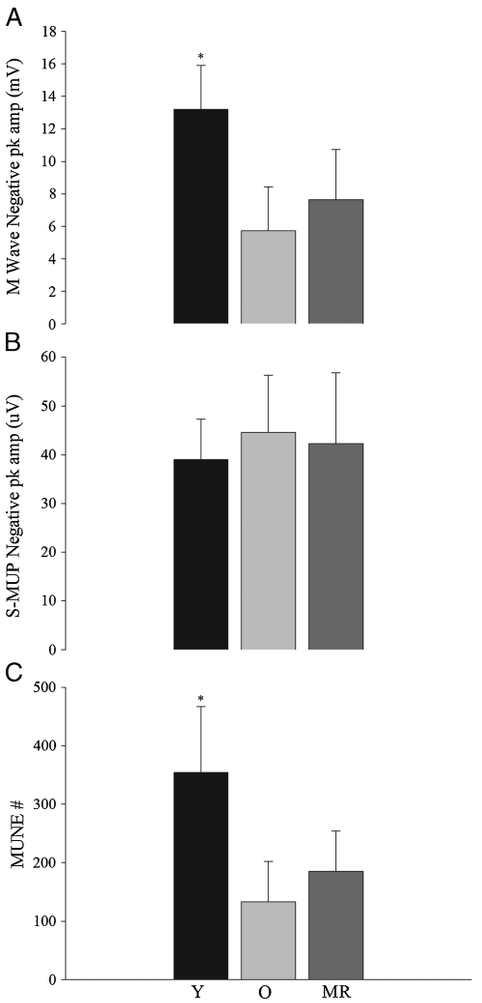

FIGURE 1—Derived MUNE (M-Wave / S-MUP). A, The negative peak amplitude of the M wave was larger for the young men (Y) compared with old (O) and master runners (MR). B, The negative peak amplitude of the mean surface MU potential (S-MUP) was similar in all groups. C, MUNE were larger for Y and did not differ between MR and O. Mean ± SD. *Significant difference between young and both old groups.

old men had similar MVC forces (P = 0.995), but both master runners (P = 0.013, 95% CI = 28.42–257.74) and old men (P = 0.016, 95% CI = 24.10–253.42) were ~40% weaker than the young men (P = 0.006, ES = 0.344; Table 1), despite all groups being capable of achieving a similar high voluntary activation (P = 0.174). Electrically evoked peak twitch force showed a main effect for age (P = 0.001, ES = 0.695). The old (P < 0.001, 95% CI = 15.07–32.50) and master runners (P = 0.001, 95% CI = 10.39–28.37) had a 65% and 53% lower twitch force than the young, respectively, whereas the master runners and old did not differ (P = 0.174, ES = 0.204). Contraction duration of the twitch was 19% longer in master runners than the young (P = 0.02, ES = 0.437, 95% CI = −49.56 to −11.41), with no other group differences (master runners and old: P = 0.132, ES = 0.221, 95% CI = −4.23 to 38.42; young and old: P = 0.275, 95% CI = −34.71 to 7.94).

MU properties. The mean RMS amplitude of the surface EMG during the constant 10% MVC contractions, normalized to the mean RMS amplitude from the MVC contractions, did not differ among groups (P = 0.166; Table 1), nor did the mean MU discharge rates (P = 0.161; Table 1). There was a main effect for group for the M wave negative peak amplitude (P < 0.001, ES = 0.578; Fig. 1A). The negative peak amplitude of the M wave was 57% lower in the old (5.73 ± 2.7 mV) than young men (13.2 ± 2.7 mV; P = 0.001, 95% CI = 4.25–10.69) and 42% lower in the master runners (7.65 ± 3.1 mV; P = 0.01, 95% CI = 2.33–8.76) than young, but the master runners and old men did not differ (Fig. 1A; P = 0.311, ES = 0.122, 95% CI = −1.29 to 5.14). M wave negative peak amplitude values from all three groups were plotted against age, and the regression analysis yielded a strong significant relationship (R^2 = 0.62, P < 0.001) between increasing age and decreasing M wave amplitude in the biceps brachii (Fig. 2). The negative peak amplitude of the mean S-MUP did not differ (P = 0.573, ES = 0.045) among groups (Fig. 1B); for master runners (42.3 ± 14.5 μV), old (44.6 ± 11.7 μV), and young (39 ± 8.3 μV) men, the regression analysis yielded no detectable effect of age on S-MUP size (R^2 = 0.02, P = 0.496; Fig. 2). There was a main effect of group (P = 0.001; ES = 0.578) for MUNE in that the old men (P = 0.001, 95% CI = 68.05–269.51) and master runners (P < 0.001, 95% CI = 120.27–321.73) had 62% and 48% lower MU numbers than the young men, respectively. There were no detectable differences in MUNE between older adults and master runners in this muscle group (P = 0.412, ES = 0.144, 95% CI = −48.51 to 152.95; Fig. 1C). With nine subjects in each group, the present study was adequately powered to detect a difference of 105 MUs or ~30% of the MU pool based on MUNE in the young. The MUNEs derived for the biceps brachii were 185 ± 69 MUs for the master runners, 133 ± 69 MUs for the old men, and 354 ± 113 MUs for the young men.

To further understand the relationship between lifelong physical activity and MUNE, two regression analyses were performed. When age was plotted against MUNE for

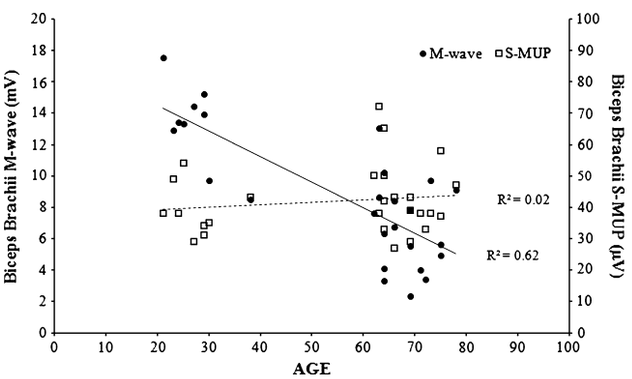

FIGURE 2—Scatterplot of age plotted against M wave (*solid line*) and S-MUP (*dashed line*) for the biceps brachii for young, old, and master runners. Biceps brachii M wave: R^2 = 0.62, r = −0.79, P < 0.001. Biceps brachii S-MUP: R^2 = 0.02, r = 0.141, P = 0.53.

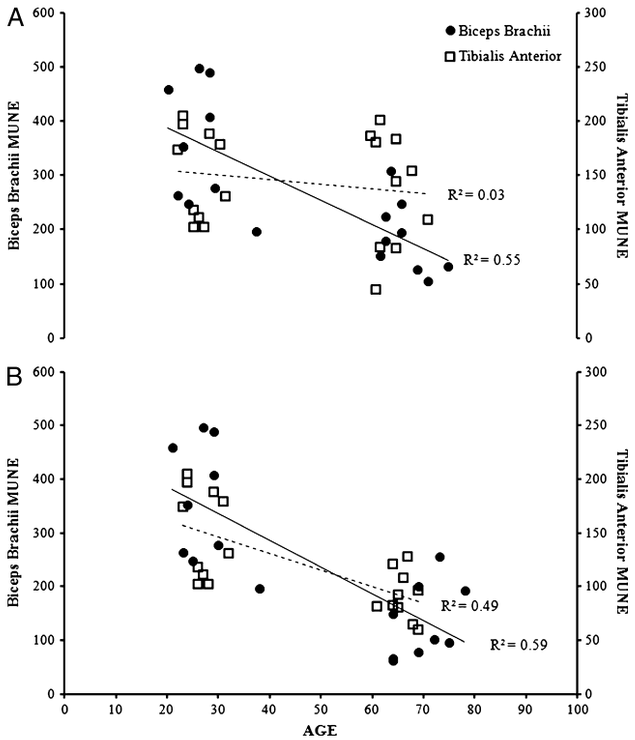

FIGURE 3—A, Scatterplot of age plotted against MUNE for the biceps brachii (*solid line*) and tibialis anterior (*dashed line*) for young and master runners. Biceps brachii: $R^2 = 0.744$, $r = -0.553$, $P < 0.001$. Tibialis anterior: $R^2 = 0.03$, $r = -0.18$, $P = 0.43$. B, Scatterplot of age plotted against MUNE for the biceps brachii (*solid line*) and tibialis anterior (*dashed line*) for young and old men. Biceps brachii: $R^2 = 0.59$, $r = -0.59$, $P = 0.0001$. Tibialis anterior: $R^2 = 0.49$, $r = -0.70$. $P = 0.001$.

the biceps brachii and tibialis anterior muscles (data from Power et al. [36]) for young and master runners, there was a strong negative relationship for the biceps brachii ($R^2 = 0.55$, $r = -0.74$, $P < 0.001$) but not the tibialis anterior ($R^2 = 0.03$, $r = -0.18$, $P = 0.430$; Fig. 3A). Meanwhile, for the old group, both the biceps brachii ($R^2 = 0.59$, $r = -0.77$, $P < 0.001$) and the tibialis anterior ($R^2 = 0.48$, $r = -0.70$, $P < 0.001$) demonstrated a typical age-related reduction in MUNE (Fig. 3B).

DISCUSSION

We tested the hypothesis that lifelong running would not provide a neuroprotective effect on functioning MUs associated with a muscle not directly loaded by running and that MUNE for the biceps brachii would demonstrate a typical age-related reduction. Our hypothesis was confirmed; the number of functioning MUs was lower in the biceps brachii of both older groups compared with the young men. This suggests that the positive effects of exercise on MU survival are dependent on direct and long-term activation of the specific MN pool exercised. In the present study, there was no detectable whole-body neuroprotective effect on MUNEs in a remote muscle group not exercised directly.

The observed age-related loss of MUNE in the biceps brachii is consistent with findings reported previously for this muscle group. Both Brown et al. (6) and Doherty et al. (13) found a similar ~50% decline in the number of functioning MUs by the seventh decade of life. The method of estimating MUs is derived by dividing the average S-MUP (sample of surface-detected MU potentials) into the M wave (sum of the entire MU pool within the muscle group). Therefore, either a decrease in the M wave amplitude or an increase in the S-MUP amplitude or both will yield a lower MUNE. In the present study, we observed a 57% and 42% lower negative peak amplitude of the M wave in the old men and master runners, respectively, compared with the young. Conversely, there was no detectable difference in the size of the S-MUP amplitude among all three groups (Fig. 1). Hence, the lower MUNE for the biceps brachii in both of the old groups was driven by those factors affecting primarily the M wave (Fig. 2) with little evidence of collateral reinnervation as suggested by minimal change in the S-MUP negative peak amplitude between the young and older groups (discussed below).

Strength, voluntary activation, and twitch properties. Accompanying the substantial loss of MUs is a progressive loss of contractile muscle mass and impaired whole muscle force generation. This is evident in the lower MVC and loss of MUs in both old groups compared with young. Despite high voluntary activation in all groups (>95%), maximal isometric strength and electrically evoked twitch force of the elbow flexors was lower in master runners and old men compared to the young. Other studies have observed similar declines in maximal strength for the elbow flexors of older adults (8,13,19,25). Typically, beyond the seventh decade of life, there is a steady (~1.5%) decline in strength per year (39), and this progressive decrease in strength may reflect a critical threshold in which the loss of MNs surpass the compensatory mechanism of collateral reinnervation, and therefore, muscle mass is reduced (31). As expected, based on previous investigations of the elbow flexors (8,13,25), the young and old men had similar twitch contractile durations; however, the master runners displayed a slowing in twitch contractile duration compared with the young but not significantly different from the old (Table 1). A slower-contracting biceps brachii muscle in the master runner group is similar to what we observed previously in the tibialis anterior (36) for this population. It is suggested that years of endurance training results in a greater percentage of Type I muscle fiber area, which have slower twitch contractile times compared to that of faster Type II muscle fibers (1,35); thus, the slower muscle contractile properties of the master runner group may represent an acclimation to lifelong endurance training or a self-selected bias for those with greater Type I fiber composition who excel in distance running in old age.

Age-related reduction in M wave. The maximum M wave negative peak amplitude was lower (almost halved) in both old groups compared with the young men (Fig. 2). Our data are consistent with the findings of Doherty et al. (13),

which showed a 34% reduction in M wave amplitude in older adults than in young. The reduction in M wave amplitude can be considered cautiously to be representative of the decrease in excitable muscle mass (40). The smaller M wave in both old groups may be due to a decrease in the number of muscle fibers secondary to age-related MU loss or a reduction in muscle mass secondary to fiber atrophy or both (9). The reduced M wave complements the MUNE by providing information about the functioning MUs but cannot differentiate between the loss of muscle fibers or atrophy, both of which are known to occur. The age-related loss of muscle mass may occur via different processes for upper and lower limbs. That is, both loss of muscle fibers and muscle atrophy contribute to sarcopenia of the lower limbs (26), whereas muscle atrophy seems to be the primary factor contributing to reduced whole muscle size of the upper limbs (25). Our results confirm previous findings suggesting an age-related loss of MUNE in the biceps brachii (13) but cannot provide insight about atrophy *per se*. Because MUs of varying sizes contribute to muscle function, the estimated loss of muscle fibers or sizes obtained via muscle biopsy does not necessarily indicate an equivalent change in MUNEs. Findings from studies involving animal preparations are equivocal regarding fiber type loss (22), whereas in humans, Type II and I muscle fibers are equally affected (27), but with a preferential atrophy (~26%) of Type II fibers compared with Type I (25,28). Because of this age-related shift in fiber type, the muscles of older adults are composed of a relatively greater percentage of Type I fibers, although MUNEs cannot distinguish between Type I and Type II.

No age-related difference in S-MUP amplitude. Indirect evidence of collateral reinnervation can be inferred from increased amplitude of the mean S-MUP in older compared with younger men. Contrary to recent evidence of age-related MU remodeling via collateral reinnervation in other muscles (30,36), here we found a similar S-MUP negative peak amplitude for all three groups (Fig. 2). Thus, our results do not support significant MU remodeling for the biceps brachii in either old group. It seems that collateral reinnervation was limited in the biceps brachii owing to similar sizes of individual MUs which did not enlarge with age. The biceps brachii, unlike the tibialis anterior, may not have "benefited" from the compensatory mechanism of collateral reinnervation in an attempt to preserve muscle mass. However, this was not the first study to suggest collateral reinnervation may be muscle dependent. An investigation of the biceps brachii (14) suggested that intrinsic changes in the muscle fibers contribute to the age-related differences. In addition, with further aging, the capacity of MUs to continue sprouting may be blunted, and this could have occurred at an earlier age in the biceps brachii as opposed to other limb muscles. A possible explanation is that reinnervation may not be keeping pace with denervation in this particular muscle group, which could explain the reduced M wave negative peak amplitude. A similar finding was reported for the soleus of young and old men (9). The explanation put forth was, if there is a reduction in muscle fiber size with a preservation of motor axons, this would inherently increase the "density" of the number of fibers within the needle's recording area and thus record a larger mean MUP amplitude. In contrast to Doherty et al. (13) who observed a significant increase in S-MUP amplitude of the biceps brachii in older adults, we did not observe any difference with age. This could be in part due to technical limitations of the method (17). The very low level voluntary contractions (i.e., 1–5 MVC) used by Doherty et al. (13) may have biased S-MUP recordings toward recording from the lowest threshold and most detectable MUs, which, presumably, would show greater collateral reinnervation than higher threshold units (16). Thus, by using a higher load of 10% MVC, in addition to the lowest threshold units, we would also be sampling from higher threshold MUs that may exhibit less collateral reinnervation. Support for this is shown by Dalton et al. (9) who observed a trend for larger S-MUPs at low-threshold contractions but similar S-MUP amplitudes at higher contraction intensities in the soleus of older adults when compared with young. Perhaps, our results based on averaging the S-MUPs from all recorded MUs masked this observation of collateral reinnervation or the current technique provides improved sampling of greater MU sizes.

Increased activity on maintained MU numbers. The neuromuscular system has a considerable degree of plasticity, altering structure and function based on the demands imposed on the system. However, the loss of a MN is an irreversible event; therefore, understanding the role of activity on preserving this basic functional unit of the neuromuscular system is of substantial importance. Lifelong physical activity has been shown to maintain the longevity of the MU (23,36). An emerging factor in the maintenance of MNs seems to be the type of activity. In two separate studies, Kanda et al. investigated the role of increased physical activity on MN survival using two distinct modalities: one involving muscle synergist ablation that effectively overloaded the remaining muscle (24) and the other involving whole-body moderate-intensity aerobic exercise (23). Their results indicate that the two types of enhanced activity resulted in different outcomes. Overloading the muscle (24) resulted in significant compensatory muscle hypertrophy, but MN numbers at the spinal cord were similar to the control limb; thus, the increased activity did not preserve the number of MNs. Conversely, those rats subjected to lifelong swimming (23) maintained MN numbers into old age. In humans, the first evidence of possible activity-dependent MU preservation was observed in the soleus (9). This unique postural muscle has the highest slow-twitch fiber composition of all limb muscles (~85%) (21) and showed a considerable resistance to MUNE loss typically associated with aging, suggesting that long-term daily activity of a fairly homogeneous slow-twitch MN pool may have helped to offset the typical age-related decline. A recent study (7) used a double-blinded immunohistological labeling protocol to identify spinal MNs of the lumbar enlargement in aged rats and found no significant difference in the number of MNs between young and old. Based on these

findings, they suggested that MN loss may be muscle type specific, which supports previous work in animal (18) and human (9) models.

The intrinsic properties of MNs may lead them to have different sensitivities to trophic factors (32), with a reduced sensitivity of high-threshold MUs. Increased long-term neuromuscular activity may increase the uptake of trophic substances (20), which, when limited, are known to cause cell death (29). Thus, the up-regulation of neurotrophic factors and sensitivity (receptors) of the MN via increased neuromuscular activity may explain the "localized" effect of exercise on MU survival. Recently, we reported that the typical 40% decrease in tibialis anterior MUNE with old age was not evident in a group of lifelong-trained master runners (36). Based on these findings, it seems that long-term moderate-intensity exercise specific to the MN pool tested attenuates the age-related degenerative changes that result in MU loss. Further evidence of muscle-specific MU survival comes from a study by Valdez et al. (38) who found that caloric restriction had whole-body effects on preservation of MNs, whereas the effects of exercise was localized to preserving only those MNs involved. The mechanisms behind MN survival could be a reduction in oxidative stress associated with a low-calorie diet, whereas the exercise could have led to a localized up-regulation of trophic factors to maintain cell health (38). A particular neurotrophin, brain-derived neurotrophic factor (BDNF), is known to increase in the central and peripheral nervous system with exercise (34) and provide a protective effect on the cell. There is a greater abundance or increase in brain-derived neurotrophic factor in muscles composed predominately of slow-twitch fibers compared with fast-twitch fibers (34). The fiber composition of human muscle is mixed, but the tibialis anterior is composed of ~70% slow twitch, whereas the biceps brachii is only ~40% slow twitch (21). This difference in fiber type could help explain the age-related decline in biceps brachii MUNE. Hence, although the biceps brachii in runners is relatively active, it may not reach a threshold of minimal activity to promote enhanced trophic transport and ameliorate MU loss when compared with the chronically active tibialis anterior of master runners (Fig. 3A).

CONCLUSIONS

Motor unit numbers were lower in older adults and master runners compared with the young in an upper limb muscle not directly overloaded by running. Although the means for MUNEs between the older adults and master runners were not significantly different in these nine subjects, we cannot eliminate the possibility of some whole-body neuroprotective effect. Clearly, the nonsignificant differences observed were not as great as those between the older adults and master runners of the locally and chronically exercised tibialis anterior. Despite the numerous health benefits of physical activity, our findings indicate that spinal MN survival is dependent on direct and long-term activation of the MN pool specific to the exercised muscle group. The possibility of some degree of whole-body neuroprotective effect requires further study.

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