Human skeletal sarcoplasmic reticulum Ca$^{2+}$ uptake and muscle function with aging and strength training

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Hunter, Sandra K., Martin W. Thompson, Patricia A. Ruell, Alison R. Harmer, Jeanette M. Thom, Tom H. Gwinn, and Roger D. Adams. Human skeletal sarcoplasmic reticulum Ca$^{2+}$ uptake and muscle function with aging and strength training. J. Appl. Physiol. 86(6): 1858–1865, 1999.—This study investigated the adaptations of skeletal muscle sarcoplasmic reticulum (SR) Ca$^{2+}$ uptake, relaxation, and fiber types in young (YW) and elderly women (EW) to high-resistance training. Seventeen YW (18–32 yr) and 11 EW (64–79 yr) were assessed for 1) electrically evoked relaxation time and rate of the quadriceps femoris; and 2) maximal rates of SR Ca$^{2+}$ uptake and Ca$^{2+}$-ATPase activity and relative fiber-type areas, analyzed from muscle biopsies of the vastus lateralis. EW had significantly slower relaxation rates and times, decreased SR Ca$^{2+}$ uptake and Ca$^{2+}$-ATPase activity, and a larger relative type I fiber area than did YW. A subgroup of 9 young (YWT) and 10 elderly women (EWT) performed 12 wk of high-resistance training (8 repetition maximum) of the quadriceps and underwent identical testing procedures pre- and posttraining. EWT significantly increased their SR Ca$^{2+}$ uptake and Ca$^{2+}$-ATPase activity in response to training but showed no alterations in speed of relaxation or relative fiber-type areas. In YWT none of the variables was altered after resistance training. These findings suggest that 1) a reduced SR Ca$^{2+}$ uptake in skeletal muscle of elderly women was partially reversed with resistance training and 2) SR Ca$^{2+}$ uptake in the vastus lateralis was not the rate-limiting mechanism for the slowing of relaxation measured from electrically evoked quadriceps muscle of elderly women.

calcium uptake; calcium adenosinetriphosphatase activity; fiber types; relaxation; strength

THE AGING PROCESS INCLUDES a slowing of time and rate of relaxation of skeletal muscle that is evident in both animals (16, 29, 34) and humans (32, 41). However, the mechanisms of age-associated slowing of muscle relaxation are not well understood.

The relaxation of a twitch contraction from skeletal muscle of aged rats has been reported to be 18–22% slower in the soleus and 43–45% slower in the extensor digitorum longus compared with in adult rats (16, 29). Relaxation times after tetanic contractions of soleus muscle of aged mice have been shown to be prolonged by 21% compared with that of young mice (34).

In elderly humans, slower times and/or rates of relaxation after a twitch or tetanic contraction of skeletal muscle have been reported for some muscle groups. In 60–70 yr olds, the ankle dorsiflexor, planar flexor, and thenar muscle groups are reported to be 20–43% slower than those in young adults (9, 10, 41), whereas in men and women over 80 yr old the ankle dorsiflexors (41) and the adductor pollicis (32) are reported to be 49–56% slower. In contrast, insignificant age-associated changes in twitch time course have been reported for the elbow flexors of elderly individuals (11, 31). To the author’s knowledge, no studies have measured the electrically evoked relaxation times and rates of the quadriceps muscle in an aged vs. young population.

Mechanisms underlying the slowing of relaxation of elderly skeletal muscle are not well understood but theoretically may involve several processes within the muscle fiber. These include 1) Ca$^{2+}$ uptake by the sarcoplasmic reticulum (SR) and cytoplasmic Ca$^{2+}$ buffers, 2) rates of dissociation of Ca$^{2+}$ from troponin, 3) the rate of cross-bridge detachment between the actin and myosin molecules, and 4) the general viscoelastic parameters of the muscle (12). Several studies have concluded that Ca$^{2+}$ uptake into the SR is the rate-limiting step in the relaxation of twitches and tetanic contractions of animal muscle (6, 17, 29) but may depend on how well developed the SR system is within the fiber (12). In aged rats it has been reported that the slowing of relaxation of skeletal muscle was primarily due to fiber-type-specific alterations in the SR volume and Ca$^{2+}$-ATPase activity (activity of the enzyme that pumps Ca$^{2+}$ into the SR) (29). Although several studies have reported reductions of up to 32% in the rate of skeletal muscle SR Ca$^{2+}$ uptake in aged rats compared with young adult rats (18, 29), other studies have reported no change in SR Ca$^{2+}$ uptake and/or Ca$^{2+}$-ATPase activity with age (15, 16, 27).

In humans, associations between SR Ca$^{2+}$ uptake and relaxation times and rates of skeletal muscle have been reported (19). However, the rate of Ca$^{2+}$ uptake into the SR has not previously been measured in the skeletal muscle of elderly individuals, nor has it been related to the slowing of relaxation observed with aging. Only one study has reported a reduced concentration of Ca$^{2+}$-ATPase in the vastus lateralis of sedentary elderly men relative to that of young men (26). Furthermore, elderly men who participated in regular lifetime strength training were found to have a similar SR Ca$^{2+}$-ATPase concentration in the vastus lateralis to that of untrained young men (26).

In the present study the association between quadriceps relaxation and rates of SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase activity in the vastus lateralis was exam-
ined in young and elderly female subjects. To further assess the relationship between age-related slowing of relaxation and SR Ca\textsuperscript{2+} kinetics, we investigated the adaptive response of SR Ca\textsuperscript{2+} uptake, SR Ca\textsuperscript{2+}-ATPase activity, and relative fiber-type areas in the vastus lateralis and relaxation of the quadriceps to a short-term, high-resistance training program in a subgroup of young and elderly women.

**MATERIALS AND METHODS**

**Subjects**

Seventeen healthy young women (YW) aged 18–32 yr and 11 healthy elderly women (EW) aged 64–79 yr participated in the study after giving informed consent (Table 1). A subgroup of 9 young women (YWT) aged 18–27 yr and 10 elderly women (EWT) aged 64–79 yr further undertook 12-wk high-resistance training of the quadriceps femoris muscle (Table 1). All protocols and procedures were approved by The University of Sydney Human Ethics Committee.

No subject had resistance training experience before the study. Subjects were medically screened to exclude cardiovascular, metabolic, and neuromuscular disease. None of the subjects was undergoing, nor had previously undergone, hormone replacement therapy.

**Experimental Overview**

Percutaneous electrically evoked contractile properties of the dominant leg quadriceps muscle group were evaluated in YW and EW. At least two familiarization sessions of electrical stimulation were conducted in the 2 wk before the initial testing session. On a separate day, resting muscle biopsies of the vastus lateralis of the same leg were performed. Muscle samples were subsequently analyzed for relative fiber-type area and maximal rates of SR Ca\textsuperscript{2+} uptake and SR Ca\textsuperscript{2+}-ATPase activity.

A subgroup of the YW and EW (YWT and EWT, respectively) subsequently performed 12-wk high-resistance strength training and were then evaluated the week after completing training, with the same muscle testing and sampling procedures applied before training.

**Measurement of Electrically Evoked Peak Rate and Time of Relaxation**

Electrically evoked contractions allow speed of relaxation to be assessed independently of volitional effort and central inhibition. Electrically stimulated measurements of rates and times of relaxation were evaluated in all young and elderly subjects (YW and EW) and then again in the EWT and YWT in the week after completion of training. Half relaxation time (RT\textsubscript{50}, time for the muscle to relax from 95 to 50% of peak tetanic force) (14) and peak rate of relaxation (PR\textsubscript{rel}) were measured from a 2-s block of submaximal 30-Hz percutaneous electrical stimulation of the dominant quadriceps. A 30-Hz tetanus was chosen for measurement of RT\textsubscript{50} and PR\textsubscript{rel} because, at higher frequencies of stimulation (e.g., 80–100 Hz), the muscle is susceptible to high-frequency fatigue, which is evident immediately on contraction and may act to prolong these variables (23).

In preparation for electrically evoked tetani at 30-Hz stimulation, the subjects were seated on a steel-framed, straight-backed adjustable chair. Chest and hip straps secured the subject to the chair to minimize upper body movement during the contractions, and the ankle of the dominant leg was strapped above the lateral malleolus to a “U”-shaped fiberglass support. The subject’s hips were flexed at 90° and the knee to an angle of 60° from full knee extension (intersection of a line linking the following external anatomic landmarks: the greater trochanter, center of the lateral femoral condyle, and center of the lateral malleolus), which corresponds to the optimal region of the knee extensor length-tension curve (28). Isometric forces were recorded via a force transducer (X-Tran 1000 N, Applied Measurement Technology; linearity of 0.03% located posteriorly with respect to the ankle support. The quadriceps muscle group was percutaneously stimulated via two 8 × 13-cm pad electrodes (86906350; Medtronic Nortech Division, San Diego, CA) placed on the proximal and distal anterolateral thigh. A high-voltage stimulator (DS7; Digitimer, Hertfordshire, UK) was employed to initiate square-wave pulses (400 V, 100 μs) with the frequency and duty cycle set by a Digitimer programmer (D4030; Digitimer). Force was amplified (Applied Measurement Technology), digitized (DT2801A analog-to-digital card, Data Technology, Malboro, MA), and sampled at a frequency of 1,000 Hz.

Contractions were evoked at maximal tolerable current levels for each subject. Two previous familiarization sessions ensured that >20% of the maximal voluntary force was achieved with evoked contractions so that a representative portion of the muscle was stimulated (2, 13). The coefficient of variation between familiarization and pretraining testing sessions (day-to-day reliability) was 2.2% for RT\textsubscript{50} and 3.1% for PR\textsubscript{rel}.

**Muscle Biopsies**

Muscle biopsies of the dominant leg vastus lateralis were performed in all YW and EW and then in the EWT and YWT the week after completion of training. Muscle biopsies of the midregion of the vastus lateralis were performed under local anesthesia (Xylocaine, 2%) by using the percutaneous needle biopsy technique (1), modified for suction. The excised muscle (~80–200 mg) was immediately divided in two portions. One portion was oriented so that the fibers were longitudinal, then frozen in cooled isopentane, and stored in liquid nitrogen at −196°C until subsequent histochemical analysis. The remaining muscle was immediately weighed; placed in buffer composed of 40 mM Tris, 0.3 M sucrose, and 5 mM dithiothreitol, pH 7.9; and homogenized on ice with a hand-held electric homogenizer (Omni 2000, Omni International) by using 3 × 15-s bursts at 60% maximal power. A portion of the homogenate was immediately frozen and stored in liquid nitrogen for subsequent analysis of maximal rate of SR Ca\textsuperscript{2+} uptake and muscle protein content. The remaining fresh homogenate was used for the immediate measurement of maximal rate of SR Ca\textsuperscript{2+}-ATPase activity.

**Muscle Protein**

Muscle homogenate protein content was determined in triplicate by using serum albumin as the standard (Precimat,
Measurement of Maximal Rate of SR Ca\(^{2+}\)-ATPase Activity

The maximal rate of SR Ca\(^{2+}\)-ATPase activity was determined in triplicate on fresh homogenate by using an established method (36). The assays were performed at 37°C by using a spectrophotometer (UV-120–02: Shimadzu, Tokyo, Japan) at 340 nm. The reaction medium consisted of 18 mM HEPEs buffer, pH 7.5, 180 mM KCl, 13 mM MgCl\(_2\), 9 mM Na\(_2\)N\(_3\), 1 mM EGTA, 0.3 mM NADH, 9 mM phosphoenolpyruvate, 22 U/ml lactate dehydrogenase, 16 U/ml pyruvate kinase, and 4 mM ATP. Ca\(^{2+}\)-ionophore A-23187 (2.5 mM) was added to the reaction medium to make the SR membrane permeable and prevent feedback inhibition of Ca\(^{2+}\)-ATPase activity. After 1.4 mg tissue wet weight of muscle homogenate was mixed with 1 ml assay buffer, the reaction was initiated with the addition of 1.1 mM CaCl\(_2\), corresponding to a free Ca\(^{2+}\) concentration (\([\text{Ca}^{2+}]_0\)) of ~121 µM (21). After 90 s, 36 mM CaCl\(_2\) was added to completely inhibit the Ca\(^{2+}\)-ATPase activity, thus allowing the measurement of basal ATPase activity, and the reaction was followed for a further 90 s. The maximal SR Ca\(^{2+}\)-ATPase activity was calculated by subtracting the basal ATPase (Ca\(^{2+}\)-independent) from the total (Ca\(^{2+}\)-dependent + Ca\(^{2+}\)-independent) ATPase activities.

Measurement of Maximal Rate of SR Ca\(^{2+}\) Uptake

Muscle homogenate maximal rate of SR Ca\(^{2+}\) uptake was measured as previously described (36). Maximal rate of muscle homogenate Ca\(^{2+}\) uptake was measured at 37°C on an Amino Bowman Series 2 luminescence spectrometer (SLM Instruments) with continuous stirring, using the fluorescent Ca\(^{2+}\) indicator 1 indo 1. The excitation wavelength was 349 nm, and the emission wavelength cycled between 410 and 485 nm (for Ca\(^{2+}\)-bound and Ca\(^{2+}\)-free indo 1, respectively) with ratiometric data collected every second. The reaction medium consisted of 20 mM HEPEs, 150 mM KCl, 10 mM Na\(_2\)N\(_3\), 6.8 mM oxalate, 5 µM N,N,N’N’-tetakis(2-pyridylmethyl)-ethane diamine, 4.5 mM MgATP, and 1 µM indo 1, pH 7.0. Addition of extra CaCl\(_2\) was not necessary because the Ca\(^{2+}\) in the assay buffers gave a starting \([\text{Ca}^{2+}]_0\) of ~1 µM. Ca\(^{2+}\) uptake was usually measured in duplicate after the addition of 50 µl muscle homogenate to 2.2 ml of reaction medium. The reaction was allowed to proceed for ~100 s before the addition of 40 µl of EGTA and 120 µl CaCl\(_2\) for calibration purposes to give final concentrations of 3.5 and 5.0 mM, respectively. The dissociation constant for the Ca\(^{2+}\)-indo 1 complex in 150 mM KCl buffer and 20 mM HEPEs, pH 7.0, was 170 nM by using precise mixtures of EGTA. For each assay\([\text{Ca}^{2+}]_0\) vs. time curve was calculated by using the equation Ca\(^{2+}\) concentration (\([\text{Ca}^{2+}]_0\)) = \(K_d [(R - R_{\text{min}})(R - R_{\text{max}})](S_0/S_2)\), where \(K_d\) is the dissociation constant for the Ca\(^{2+}\)-indo 1 complex; \(R_{\text{max}}\) and \(R_{\text{min}}\) are maximal and minimal ratios (R), respectively; \(S_2\) is the fluorescence of the solution at 485 nm with added EGTA; and \(S_0\) is the fluorescence of the solution at 485 nm with saturating Ca\(^{2+}\) added (22). The [Ca\(^{2+}\)] curve was then smoothed, and a first-derivative curve was generated as described previously (36). The resulting maximal rate of Ca\(^{2+}\) uptake was normalized to the same [Ca\(^{2+}\)] (1 µM) and corrected for protein content. Results obtained were expressed as both per gram of muscle wet weight and per milligram of protein. However, because the results were the same, only data corrected for protein content are presented here.

Histochemistry

Frozen muscle samples were embedded in partially frozen mounting medium (OCT, TissueTek, 4583) on a cork disk and oriented vertically to allow fibers to be cut into transverse sections (10 µm thick) in a cryostat (4451 TissueTek II, Miles) at ~20°C. Routine myofibrillar ATPase histochemical analysis was performed after preincubation at pH values of 4.3, 4.6, and 10.3 (7). Two fiber types were delineated (types I and II) on the basis of their staining intensities. Pretraining and posttraining samples from those subjects who performed high-resistance training (YWT and EWT) were sectioned on the same day and assayed together for myofibrillar ATPase activity to eliminate interassay variability.

Photomicrographs (×10) were taken of the sections by using a Leitz photomicroscope (Leitz, Wetzlar, Germany). Images from negatives were scanned into digital form and were analyzed for fiber-type percentages and fiber cross-sectional area (CSA). These analyses were conducted by using planimetric techniques on Image Pro Plus software (Media Cybernetics 1996, Silver Spring, MD). The mean number of fibers measured to determine fiber CSA per sample was 111 ± 6 (type I) and 130 ± 8 (type II). Only those fibers without artifact, with distinct cell borders, and no tendency toward longitudinal cuts were included in the analysis for CSA (4). Percent fiber composition was calculated from a mean count of 648 ± 28 fibers/sample (range 385–1,070). To determine the mean coefficient of variation, 2 counts were performed on 54 images. The mean coefficient of variation between the first and second count was 3.5% for all fiber types (2.1% (type I) and 4.2% (type II)). Relative (percent) fiber-type area of each sample was calculated by using the mean percent composition and mean CSA of each fiber type.

Physical Activity Levels

Present physical activity levels were assessed by using the National Heart Foundation Prevalence Risk Study Questionnaire (Q22–25) (33). By using information from this questionnaire, each subject was rated on a six-point physical activity scale, which ranged between one (sedentary) and six (extremely active).

High-Resistance Training Protocol

The YWT and EWT groups performed 12 wk of supervised high-resistance training of the quadriceps by using a pin-loaded variable-resistance knee extension machine (Kolossal Fitness Systems, Sydney, Australia). The women performed three sets of bilateral eight-repetition-maximum (8RM) knee extensions per session, with three sessions conducted per week. An 8RM weight was defined as the load that the subject was able to concentrically lift on eight successive occasions, with failure on the ninth lift. A successful lift was objectively measured by setting a target height to which the subject lifted the bar at the completion of each concentric lift. Each concentric knee extension lift was conducted over an 80° range of knee motion that remained constant for each subject throughout the training. Subjects were instructed at each training session to lift until failure. Reassessment and subsequent adjustment of the 8RM training weight were performed throughout the training program to maintain the training...
SR Ca\textsuperscript{2+} UPTAKE, AGING, AND STRENGTH TRAINING

Table 2. RT\textsubscript{1/2} and PR\textsubscript{rel} from 30-Hz tetani of the quadriceps, maximal rates of Ca\textsuperscript{2+}-ATPase activity and Ca\textsuperscript{2+} uptake, and percent area of fiber types in young and elderly vastus lateralis.

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<th>YW</th>
<th>EW</th>
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<tr>
<td>RT\textsubscript{1/2}, ms</td>
<td>45 ± 1</td>
<td>59 ± 2\textdagger</td>
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<tr>
<td>PR\textsubscript{rel}, %/ms</td>
<td>-5.1 ± 0.1</td>
<td>-3.9 ± 0.2\textdagger</td>
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<td>Ca\textsuperscript{2+} uptake, nmol·mg\textsuperscript{-1}·protein\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>10.5 ± 0.6</td>
<td>7.1 ± 0.8\textdagger</td>
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<tr>
<td>Ca\textsuperscript{2+}-ATPase activity, nmol·mg\textsuperscript{-1}·protein\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>80.3 ± 4.1</td>
<td>51.1 ± 3.88\textdagger</td>
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<tr>
<td>Type I area, %</td>
<td>54.3 ± 2.2</td>
<td>60.9 ± 2.3\textdagger</td>
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<tr>
<td>Type II area, %</td>
<td>45.7 ± 2.2</td>
<td>39.1 ± 2.3\textdagger</td>
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Values are means ± SE of 17 YW and 11 EW. RT\textsubscript{1/2}, half relaxation time; PR\textsubscript{rel}, peak relaxation rate. Significant difference between YW and EW: *P < 0.05; †P < 0.01; ‡P < 0.0001.

stimulus. Subjects were firmly secured by a seat belt passing over their hips, and they were not permitted to use their upper body to secure their position in the seat. All training sessions were preceded by a warm-up that consisted of 1) stationary cycling at 25–50 W for 10 min and 2) lifting light weights.

Measurement of 1RM Strength

Two-legged 1RM knee extension strength was assessed in the week before and after 12-wk high-resistance training, using the same pin-loaded variable-resistance knee extension machine (Kolossal Fitness Systems) for training. 1RM was considered to be the amount of weight capable of being lifted once only. When a subject failed to lift a load on two separate occasions, the previous weight lifted was designated as the 1RM. The lifting conditions were identical to that of the 8RM, i.e., use of an objective target, use of 80° range of motion, and no use of arms. A similar warm-up to that adopted during training was used before assessment of the 1RM, with a 2-min break between successive lifts.

Statistical Methods

Independent two-tailed t-tests were used to analyze differences between means of variables for the YW and EW. Repeated-measures ANOVA was used to assess the effect of high-resistance training on dependent variables in the YWT and EWT groups. Where a significant interaction (P < 0.05) between groups was found, paired t-tests were used to confirm the differences as a result of training. Pearson product-moment correlations were used to assess the relationship between variables. All data are expressed as means ± SE, and significance was accepted at P < 0.05.

RESULTS

Electrically Evoked Peak Rates and Time of Relaxation

Young vs. elderly. The mean RT\textsubscript{1/2} of EW quadriceps muscle was prolonged (P < 0.0001) and PR\textsubscript{rel} was slower for YW (P < 0.0001) by 31% for both variables (Table 2). PR\textsubscript{rel} and RT\textsubscript{1/2} were highly correlated (r = 0.92, P < 0.0001).

High-resistance training. The RT\textsubscript{1/2} of YWT (45 ± 1 to 45 ± 1 ms, pre- vs. posttraining, P > 0.05) and EWT (60 ± 3 to 58 ± 2 ms, pre- vs. posttraining, P > 0.05) did not change in response to 12-wk high-resistance training. Similarly, the PR\textsubscript{rel} of YWT (−5.0 ± 0.1 to −5.0 ± 0.1%/ms, pre- vs. posttraining, P > 0.05) and EWT (−3.8 ± 0.2 to −4.0 ± 0.1%/ms, pre- vs. posttraining, P > 0.05) remained unaltered after high-resistance training.

Maximal Rate of SR Ca\textsuperscript{2+} Uptake and SR Ca\textsuperscript{2+}-ATPase Activity

Young vs. elderly. The EW had lower maximal rates of SR Ca\textsuperscript{2+}-ATPase activity (P < 0.0001) and SR Ca\textsuperscript{2+} uptake (P < 0.01) than did YW (Table 2). Compared with the YW, the EW showed a 37% reduction in the maximal rate of SR Ca\textsuperscript{2+}-ATPase activity and a 33% reduction in SR Ca\textsuperscript{2+} uptake.

The association between SR Ca\textsuperscript{2+} uptake and SR Ca\textsuperscript{2+}-ATPase activity was not different between age groups, with the mean ratio of SR Ca\textsuperscript{2+} uptake to SR Ca\textsuperscript{2+}-ATPase activity being 0.14 ± 0.01 for both YW and EW. Pooled data of YW and EW showed a significant association between rates of SR Ca\textsuperscript{2+}-ATPase activity and SR Ca\textsuperscript{2+} uptake (r = 0.70, P < 0.001). Pooled data of YW and EW indicated that SR Ca\textsuperscript{2+}-ATPase activity and SR Ca\textsuperscript{2+} uptake were significantly associated with RT\textsubscript{1/2} (r = −0.61, P < 0.01 and r = −0.60, P < 0.01, respectively) and PR\textsubscript{rel} (r = −0.64, P < 0.01 and r = −0.41, P < 0.05, respectively).

High-resistance training. SR Ca\textsuperscript{2+} Uptake. A significant interaction between YWT and EWT for the maximal rate of Ca\textsuperscript{2+} uptake (P < 0.05) was found as a result of training. Pairwise comparison indicated that the maximal rate of SR Ca\textsuperscript{2+} uptake in EWT was increased after training (23%, P < 0.05), whereas that of the YWT did not change with training (P > 0.05) (Fig. 1). As a consequence of the elevation in maximal rates of SR Ca\textsuperscript{2+} uptake in the EWT, the maximal rates were not statistically different from those of the YWT after training (P > 0.05).

SR Ca\textsuperscript{2+}-ATPASE ACTIVITY. Although the group interaction for training and the maximal rate of SR Ca\textsuperscript{2+}-ATPase activity did not reach the P < 0.05 significance
criterion, ($P = 0.07$), pairwise comparisons showed that the maximal rate of SR Ca$_{2}^{+}$-ATPase activity was increased after training (26%, $P < 0.05$) for EWT, whereas there was no change for the YWT ($P > 0.05$) (Fig. 2). Despite the increase after training, the maximal rate of SR Ca$_{2}^{+}$-ATPase activity in EWT remained significantly lower than that in YWT ($P < 0.05$).

The ratio of SR Ca$_{2}^{+}$ uptake to SR Ca$_{2}^{+}$-ATPase activity remained constant in the YWT and EWT. Posttraining, the mean ratio of SR Ca$_{2}^{+}$ uptake to SR Ca$_{2}^{+}$-ATPase activity was not different between groups (EWT $0.14 \pm 0.01$ and YWT $0.12 \pm 0.01$, $P > 0.05$) and did not differ from pretraining results in either the EWT ($0.14 \pm 0.01$, $P > 0.05$) or YWT ($0.14 \pm 0.01$, $P > 0.05$) group.

Pooled pre- and posttraining data in YWT and EWT indicated that $RT_{10}$ and $PR_{rel}$ remained associated with SR Ca$_{2}^{+}$-ATPase activity ($r = -0.53$, $P < 0.001$ and $r = -0.60$, $P < 0.001$, respectively) and SR Ca$_{2}^{+}$ uptake ($r = -0.41$, $P < 0.01$ and $r = -0.51$, $P < 0.001$, respectively). However, there were no significant associations between the change in $RT_{10}$ or change in $PR_{rel}$ with a change in either SR Ca$_{2}^{+}$-ATPase activity or SR Ca$_{2}^{+}$ uptake that occurred in response to high-resistance training.

Percent Fiber-Type Area

Young vs. elderly. The relative area of type I fibers in the EW was greater than that in the YW ($P = 0.05$), and the relative area of type II fibers in the EW was less than that in the YW ($P = 0.05$) (Table 2).

Pooled data of YW and EW showed that the relative area of type I fibers was associated with SR Ca$_{2}^{+}$ uptake ($r = -0.49$, $P < 0.02$); $RT_{10}$ ($r = 0.41$, $P < 0.05$); and $PR_{rel}$ ($r = 0.44$, $P < 0.05$).

High-resistance training. Training had no effect on the relative type I or type II fiber areas of the YWT or EWT. Pre- and posttraining, the relative type I area of the YWT ($n = 7$) was $56.2 \pm 2.6$ and $53.9 \pm 3.4\%$, respectively, whereas that of the EWT was $61.2 \pm 2.5$ and $60.2 \pm 3.7\%$, respectively.

Physical Activity Levels

YW were more physically active ($3.9 \pm 0.3$) than EW ($2.0 \pm 0.3$, $P < 0.001$). Of those subjects who completed the high-resistance training, the YWT ($4.1 \pm 0.4$) were also more physically active than the EWT ($1.9 \pm 0.3$, $P < 0.001$).

Strength (1RM and 8RM)

1RM strength. Before training the EWT were able to lift 51% of the 1RM load of the YWT ($P < 0.0001$). High-resistance training resulted in a significant increase in the 1RM strength of EWT ($41 \pm 3$ to $57 \pm 4$ kg, pre- vs. posttraining, $P < 0.0001$) and for YWT ($81 \pm 6$ to $104 \pm 4$ kg, pre- vs. posttraining, $P < 0.0001$) (Fig. 3).

8RM training load. The 8RM load of the EWT increased from $28 \pm 2$ (pretraining) to $46 \pm 3$ kg (posttraining) ($P < 0.0001$). The YWT increased their 8RM from $61 \pm 4$ (pretraining) to $86 \pm 3$ kg (posttraining) ($P < 0.0001$). The relative improvements in 1RM and 8RM strength were similar in YWT and EWT, demonstrated by a lack of significance in the groups by training interaction ($P > 0.05$).

DISCUSSION

SR Ca$_{2}^{+}$ uptake, muscle relaxation, and relative fiber-type area of the quadriceps were compared in young and elderly women, and the responses to short-term resistance training were investigated. The major findings were that 1) the elderly women had lower maximal rates of SR Ca$_{2}^{+}$ uptake and SR Ca$_{2}^{+}$-ATPase activity in resting vastus lateralis compared with the young women; 2) the maximal rates of SR Ca$_{2}^{+}$ uptake and SR Ca$_{2}^{+}$-ATPase activity in the vastus lateralis of elderly women were augmented after high-resistance strength training; and 3) the elderly women had slower rates and times of quadriceps relaxation compared with the young women, and these were not altered in

Fig. 2. Vastus lateralis maximal rate of SR Ca$_{2}^{+}$-ATPase activity in YWT ($n = 9$) and EWT ($n = 10$) pre- and post-12-wk high-resistance training. Values are means ± SE. * Significant effect of training, $P < 0.05$.

Fig. 3. One repetition maximum (1RM) knee extension of young women (YWT, $n = 9$) and elderly women (EWT, $n = 10$), pre- and post-12-wk high-resistance training. Shown are individual data of subjects.
either group after resistance training. These findings indicate that the maximal rate of SR Ca$^{2+}$ uptake in the vastus lateralis was not the rate-limiting mechanism for the slowing of relaxation measured from electrically evoked quadriceps muscle of the elderly women. Furthermore, short-term high-resistance training of equal intensity to that performed by the elderly women did not change the maximal rate of SR Ca$^{2+}$ uptake or SR Ca$^{2+}$-ATPase activity in vastus lateralis muscle of the young women.

**Reductions in SR Ca$^{2+}$ Uptake and SR Ca$^{2+}$-ATPase Activity of Elderly Women are Reversed With Resistance Training**

It was found that human vastus lateralis muscle of elderly women had a depressed maximal rate of SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase activity compared with that in young women. The reduction was the same whether or not the SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase activity were normalized for protein concentration of the sample. Furthermore, the ratio of SR Ca$^{2+}$ uptake to SR Ca$^{2+}$-ATPase activity was not altered with increased age, indicating that there was no uncoupling or inefficiency in the SR Ca$^{2+}$ uptake within the muscle fibers of vastus lateralis in the elderly compared with that of the young subjects. The coupled reduction of SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase activity in the muscle of the elderly women suggests there was a structural alteration of the SR with increasing age. This alteration may have included a reduction in the SR Ca$^{2+}$-ATPase pump concentration, which is consistent with the findings of Klitgard et al. (26), or an alteration in the expression of the SR Ca$^{2+}$-ATPase pump isoform.

SR Ca$^{2+}$ uptake in the vastus lateralis of the elderly women was upregulated by a short-term program of high-resistance training to values approaching that of the young women, thereby demonstrating the plasticity of skeletal muscle SR in sedentary elderly women. Although 1) there was an association between the relative area of type I fibers and SR Ca$^{2+}$ uptake ($r = -0.49$ and 2) single-fiber studies have shown that human type I fibers have a faster rate of SR Ca$^{2+}$ uptake than that of type I fibers independent of SR volume (37), the training-induced increased rates of SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase activity in the elderly muscle were not caused by changes in the relative fiber-type area in this present study because this variable was altered significantly with training. The increases in maximal rates of SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase activity observed in the present study after 12-wk resistance-strength training (23 and 26%, respectively) were of a magnitude similar to the greater SR Ca$^{2+}$-ATPase concentration (31%) found in the vastus lateralis of lifetime-strength-trained elderly men compared with sedentary elderly men (26). Although an increase in SR Ca$^{2+}$-ATPase concentration may serve as an explanation for the increased SR Ca$^{2+}$ uptake after training, changes in SR Ca$^{2+}$-ATPase isoform expression may also account for the increase in SR Ca$^{2+}$ uptake in the elderly women. In rat skeletal muscle SR Ca$^{2+}$-ATPase activity and fast Ca$^{2+}$-ATPase pump isoform [sarc(endo)plasmic reticulum Ca$^{2+}$-ATPase isoform 1 (SERCA1)] have been shown to increase in response to hindlimb suspension (38), which is inconsistent with the findings of this human study. Furthermore, overload of animal skeletal muscle has been reported to reduce SR Ca$^{2+}$ uptake, SR Ca$^{2+}$-ATPase activity, and SERCA1 (24, 39). Further work is required to establish whether the increase in SR Ca$^{2+}$-ATPase activity and SR Ca$^{2+}$ uptake found in the skeletal muscle of elderly humans in response to resistance training is due to an increase in Ca$^{2+}$-ATPase concentration and/or an alteration of isoform expression.

In contrast to the elderly women, in the young, relatively active women the maximal rates of SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase activity of vastus lateralis were not altered after resistance training. Before training, the maximal SR Ca$^{2+}$ uptake of the vastus lateralis of the young women in this study (10.5 nmol·mg$^{-1}$protein·min$^{-1}$) was similar to that reported in young men (10.4 nmol·mg$^{-1}$protein·min$^{-1}$) (5). The failure of the SR of skeletal muscle in the young women to adapt to short-term high-resistance training was consistent with a previous study that reported no alteration in SR Ca$^{2+}$-ATPase activity in the vastus lateralis of young men after 12-wk high-resistance training (20). It is important to note that the absence of adaptation of SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase found in young subjects in the latter study and the present one may be specific to the training dosages and duration imposed. The effects of a more intense training schedule and/or longer training duration require further investigation.

In the present study, the elderly subjects were significantly less active than the young women. It may be speculated that the initial difference in physical activity levels between the young and elderly women accounted for the lower rates of SR Ca$^{2+}$ uptake in the muscle of elderly women and also for the different adaptive responses of the young and elderly SR to resistance training. In support of this view, the SR Ca$^{2+}$-ATPase pump concentration was found to be reduced in sedentary elderly men but not in those elderly men who had performed lifetime strength training compared with that in young untrained men (26). Interestingly, in the same study, endurance-trained elderly men were also deficient in SR Ca$^{2+}$-ATPase pump concentration compared with both young untrained men and strength-trained elderly men (26). These results imply that specific strength-type activities are able to reverse the reductions in SR Ca$^{2+}$ kinetics of elderly muscle.

**Slowing of Aged Muscle and SR Ca$^{2+}$ Uptake**

Relaxation times and rates of the quadriceps in the elderly women were 31% slower than that in the young women. These findings are consistent with other studies that have used electrically evoked twitch or tetanic contractions to demonstrate a slowing of relaxation in elderly human muscle (9, 10, 32, 41).
This is the first study to investigate the relationship among muscle relaxation, the rate of SR Ca$^{2+}$ uptake, and relative fiber-type areas in human muscle. Despite a statistically significant association between SR Ca$^{2+}$ uptake and relaxation time and rates, the maximal rate of SR Ca$^{2+}$ uptake from the vastus lateralis could not have been the rate-limiting process that determined the slowing of relaxation time and rate of the quadriceps muscle in the elderly women, because the 23% increase in SR Ca$^{2+}$ uptake in the elderly vastus lateralis after training failed to alter the measured relaxation times of the quadriceps. In response to high-resistance training of elderly muscle, in other studies relaxation times from a twitch contraction have been either slowed by 14% (8) or have shown no change (25, 35).

The dissociation between relaxation time and rates and SR Ca$^{2+}$ uptake indicated that a mechanism(s) other than the maximal rate of SR Ca$^{2+}$ uptake governed the slowing of relaxation times and rates of the quadriceps in the elderly women. Other rate-limiting processes of relaxation within the fiber theoretically may include the uptake of Ca$^{2+}$ by cytoplasmic buffers; the rate of dissociation of Ca$^{2+}$ from troponin; the rate of cross-bridge detachment between the actin and myosin molecules; and/or the general viscoelastic properties of the muscle (12). However, the dissociation may have been related to the limitations inherent in comparing the in vitro physiological properties of a small sample of muscle excised from the human vastus lateralis with the in vivo functional characteristics measured in the four different muscles of the intact quadriceps. For obvious practical reasons, the SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase activity were measured in vitro and the relaxation was measured in vivo. There may be limitations in comparing relaxation time that was measured in milliseconds with an in vitro measurement of Ca$^{2+}$ uptake that was measured over 60 s. Furthermore, the measurement of relaxation in vivo is capable of being influenced by factors other than the speed of the contractile elements, such as musculotendinous compliance. High-resistance training of elderly men has previously been reported to prolong the relaxation time of a twitch contraction (8). This may have been due to a stiffer elderly musculotendinous complex (3, 40) becoming more compliant in response to high-resistance training (3). A more compliant musculotendinous complex after high-resistance training would oppose any increase in the intrinsic speed of relaxation that resulted from increased maximal SR Ca$^{2+}$ uptake.

In conclusion, plasticity of the skeletal muscle SR of sedentary elderly women was demonstrated in response to short-term high-resistance training. The depressed maximal rate of SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase activity of elderly vastus lateralis was reversed, in part, after high-resistance training, whereas the SR Ca$^{2+}$ uptake and SR Ca$^{2+}$-ATPase activity of young skeletal muscle remained unchanged. The relaxation rate of the quadriceps muscle group in the elderly women was slower than that in the young women. However, the increased rate of SR Ca$^{2+}$ uptake of elderly vastus lateralis measured in vitro after high-resistance training did not result in an increased speed of relaxation of the quadriceps measured in vivo, nor was there any change in the relative area of type I or II fibers of the vastus lateralis as a consequence of training. These findings suggest that maximal rate of SR Ca$^{2+}$ uptake of the vastus lateralis was not the rate-limiting process governing the slowing of relaxation in the human quadriceps muscle of elderly women.

The authors thank Diane Eager for technical assistance. Furthermore, the authors acknowledge the work, enthusiasm, and inspiration of Prof. John Robert Sutton, who passed away on February 7, 1996, for his expertise and contribution to the collection of muscle samples in this study and the inspiration and motivation he provided.

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Received 1 April 1998; accepted in final form 19 January 1999.

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