Effects of fatigue and training on sarcoplasmic reticulum Ca\(^{2+}\) regulation in human skeletal muscle

JIA L. LI, XIAO N. WANG, STEVE F. FRASER, MICHAEL F. CAREY, TIM V. WRYGLEY, AND MICHAEL J. MCKENNA

1School of Human Movement, Recreation and Performance, and 2School of Life Sciences and Technology, Centre for Rehabilitation, Exercise and Sports Science, Victoria University of Technology, Melbourne, 8001 Victoria, Australia

Received 5 July 2000; accepted in final form 15 October 2001

Effects of fatigue and training on sarcoplasmic reticulum Ca\(^{2+}\) regulation in human skeletal muscle. J Appl Physiol 92: 912–922, 2002. First published October 19, 2001; 10.1152/japplphysiol.00643.2000.—Little is known about fatigue and training effects on sarcoplasmic reticulum (SR) function in human muscle, and we therefore investigated this in eight untrained controls (UT), eight endurance-trained (ET), and eight resistance-trained athletes (RT). Muscle biopsies (vastus lateralis) taken at rest and after 50 maximal quadriceps contractions (180°/s, 0.5 Hz) were analyzed for fiber composition, metabolites and maximal SR Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase activity. Fatigue reduced (P < 0.05) Ca\(^{2+}\) release (42.1 ± 3.8%, 43.4 ± 3.9%, 31.3 ± 6.1%), Ca\(^{2+}\) uptake (43.0 ± 5.2%, 34.1 ± 4.6%, 28.4 ± 2.8%), and Ca\(^{2+}\)-ATPase activity (38.6 ± 4.2%, 48.5 ± 5.7%, 29.6 ± 5.6%), in UT, RT, and ET, respectively. These decreases were correlated with fatigability and with type II fiber proportion (P < 0.05). Resting SR measures were correlated with type II proportion (r ≥ 0.51, P < 0.05). ET had lower resting Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase (P < 0.05) than UT and RT (P < 0.05), probably because of their lower type II proportion; only minor effects were found in RT. Thus SR function is markedly depressed with fatigue in controls and in athletes, is dependent on fiber type, and appears to be minimally affected by chronic training status.

IN RECENT YEARS, accumulating evidence has implicated altered intracellular Ca\(^{2+}\) regulation as a major contributor to fatigue. Studies utilizing single fibers isolated from mice, frog, and toad muscles have demonstrated impaired sarcoplasmic reticulum (SR) Ca\(^{2+}\) release with fatigue, with a consequent decline in tetanic cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\text{cyst}\)) and muscle force (1, 13, 30, 54, 55). The mechanisms for impaired SR Ca\(^{2+}\) release with fatigue remain incompletely understood. However, these most likely include cytosolic accumulation of Ca\(^{2+}\), Mg\(^{2+}\), IMP, and AMP; localized depletion of glycogen and ATP; and SR luminal Ca\(^{2+}\cdot\text{P}\), precipitation (3, 11, 16, 18, 30, 42, 48). Despite these recent advances in understanding in other species, little is known about the effects of fatigue on SR Ca\(^{2+}\) release in human muscle.

In human skeletal muscle, it is not possible to study SR Ca\(^{2+}\) regulation in intact single fibers, and thus recent studies have utilized crude muscle homogenate preparations (6, 20, 23, 25, 27, 45). Studies investigating SR function in human muscle with exercise have demonstrated depressed SR Ca\(^{2+}\) uptake and/or Ca\(^{2+}\)-ATPase activity with prolonged submaximal contractions (6, 23, 52), a decrease in SR Ca\(^{2+}\) uptake after intense contractions (20, 25, 27), and, recently, depressed SR Ca\(^{2+}\) release after 7 min of intense fatiguing knee extensor muscle contractions (27). In the latter study, the torque decline was near maximal by midexercise, although muscle biopsies for SR measures were not taken until the cessation of exercise several minutes later (27). This raises the possibility of a different time course in the decline in Ca\(^{2+}\) release and muscle torque with fatigue. Furthermore, the training status of their subjects was not specified, and this may potentially have an important bearing on the decline in SR function. Surprisingly, they found no effects of repeated maximal contractions on SR Ca\(^{2+}\)-ATPase activity, despite a reduction in SR Ca\(^{2+}\) uptake (27), a dissociation not evident in earlier human exercise studies (6, 23, 52). The reason for this discrepancy is unclear and worthy of further investigation. Therefore, the first aim of this study was to investigate the effects of fatigue induced by repeated maximal contractions on each of SR Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase activity in human skeletal muscle with biopsy sampling coincident with the rapid decline in peak torque.

Resistance and endurance training induce fundamentally different physiological and muscular performance outcomes (e.g., 5, 22, 37), and their effects on SR function are therefore of interest. However, such studies are sparse and yield conflicting results. Ryano...
receptor (RyR) binding was reduced in long-term resistance-trained elderly men (32), whereas SR Ca$^{2+}$-ATPase protein expression and Ca$^{2+}$ uptake were reduced in hypertrophied rat muscle (31). In contrast, neither SR Ca$^{2+}$-ATPase activity (23) nor content (32) was changed after short- or long-term resistance training in humans, whereas both SR Ca$^{2+}$ uptake and Ca$^{2+}$-ATPase activity were elevated with resistance training in elderly, but not young, women (28). Endurance training in rats induces changes in muscle SR function or SR proteins consistent with an increased expression of oxidative fibers [see review (39)]. Surprisingly, the effects of endurance training on SR function in young adults are unknown, and little is known about whether training protects against deteriorating SR function with fatigue. In humans, resistance training attenuated the decline in SR Ca$^{2+}$-ATPase activity with prolonged submaximal exercise (23), and endurance-trained rats displayed a higher Ca$^{2+}$-ATPase activity at exhaustion than untrained rats (4). No studies have investigated whether resistance or endurance training in humans alters Ca$^{2+}$ release in resting muscle or attenuates the reduction in Ca$^{2+}$ release with fatigue. Therefore, the second aim of this study was to determine the effects of chronic resistance and endurance training on SR Ca$^{2+}$ release, Ca$^{2+}$ uptake, and Ca$^{2+}$-ATPase activity in human skeletal muscle, both at rest and after fatiguing maximal contractions.

Three hypotheses were tested in human skeletal muscle: 1) that fatigue, maximal contractions would depress SR Ca$^{2+}$ release and Ca$^{2+}$-ATPase activity; 2) that chronic endurance-trained and resistance-trained athletes would exhibit lower rates of SR Ca$^{2+}$ release, Ca$^{2+}$ uptake, and Ca$^{2+}$-ATPase activity in resting muscle; and 3) that the decline in muscle SR function with fatigue would be attenuated in both endurance-trained and resistance-trained athletes.

**METHODS**

**Subjects**

Eight untrained subjects (UT) and eight resistance-trained (RT) and eight endurance-trained (ET) athletes participated in the study. The UT subjects were recreationally active but were not well-trained and did not participate in regular sporting activities. The ET and RT athletes had been training continuously for at least 2 yr. During this period, the ET athletes had performed running and/or cycling endurance training for at least 5–6 h/wk and had a peak oxygen consumption ($V_{O2peak}$) exceeding 60 ml·min$^{-1}$·kg$^{-1}$. The RT subjects trained with heavy weights, including knee extension and squatting exercises, typically performing three sets, six to eight repetitions for at least 1 h, and at least three sessions/wk. All were able to perform a power-lifting-style squat exercise with free weights at least 1.5 times their body mass. No differences in age, height, or body mass were found between the three groups (Table 1). Subcutaneous skinfold thickness was measured at eight sites (triceps, biceps, subscapular, midaxilla, suprailiac, abdominal, anterior thigh, and medial calf) and summed, and maximal thigh circumference was also determined. Before commencing the study, each subject gave written, informal consent. The study was approved by the Victoria University of Technology Human Research Ethics Committee.

**Maximal Aerobic Power**

Each subject refrained from exercise, alcohol, and caffeine consumption for 24 h before each exercise test. An incremental exercise test (25 W/min, 60 rpm except ET, 80 rpm) was performed on an electrically braked cycle ergometer (Lode, Groningen, Netherlands) to determine their $V_{O2peak}$. Subjects breathed through a Hans-Rudolph three-way nonre-breathing valve, with expired air passed through flexible tubing into a mixing chamber; expired volume was measured using a ventilometer (KL Engineering, Sunnyvale, CA); mixed expired $O_2$ and $CO_2$ contents were analyzed by rapidly responding gas analyzers (Applied Electrochemistry S-3A $O_2$ and CD-3A $CO_2$, Ametek, PA). The gas analyzers were calibrated immediately before and rechecked after each test, by using commercially prepared gas mixtures. The ventilometer was calibrated before each test with a standard 3-liter syringe. Heart rate was recorded from the electrocardiogram.

**Muscle Function Assessment**

**Torque-velocity relationship.** The peak muscle-generated torque was measured during maximal concentric knee extension contractions at limb angular velocities of 60, 120, 180, 240, 300, and 360°/s on a Biodex isokinetic dynamometer (Biodex Medical Systems, Shirley, NY). Peak torque at each velocity was used to construct a torque-velocity relationship. Before they were tested, subjects warmed up by cycling at 50 W for 3 min and were then strapped to the Biodex chair with belts across the hips, chest, and leg to stabilize the upper body and thigh. After submaximal familiarization contractions for each velocity, subjects performed two practice maximal repetitions, had 1 min of rest, and then performed three maximal dynamic repetitions, each separated by a 2-min rest. The robust reliability of isokinetic measures of muscular strength has been demonstrated in numerous studies (57).

**Fatigue test.** Subjects performed two trials of a test designed to induce rapid muscular fatigue of the knee extensor muscles. The first was conducted without invasive procedures on the Biodex dynamometer, and the second was conducted on a Cybex isokinetic dynamometer (Cybex II, Lumex, Ronkonkoma, NY) ≈7 days later and included both pre- and postexercise muscle biopsies. Separate dynamometers were used for the muscle fatigue and torque-velocity tests for practical reasons. The fatigue test comprised 50 repetitions of maximal concentric knee extension at 180°/s and at 0.5 Hz, with intervening passive knee flexion, as modified from Thor-
stensson and Karlsson (51). This test was chosen because maximal voluntary contractions would be expected to recruit the entire motoneuronal pool and thus provide a maximal exercise. Five to seven repeated exhaustive contractions would be expected to maximize any fatigue effects on SR function. The peak torque during the test was calculated as the mean of the 5 strongest in the first 10 contractions. The final peak torque during the test was calculated as the mean of the 5 weakest in the final 10 contractions. The fatigue index (FI) was calculated as the percent decline in torque from peak to final contractions. The FI results were compared for the noninvasive and invasive trials and found to be reproducible (n = 24, r = 0.77, P < 0.0001), consistent with previous reports of a low method error (51). Each subject refrained from exercise, alcohol, and caffeine consumption for 24 h before the test.

Muscle Biopsy Sampling and Analyses

A muscle biopsy was taken at rest and immediately after cessation of the fatigue test. After injection of a local anesthetic into the skin and fascia (2% Xylocaine), two small separate incisions were made in the midportion of the vastus lateralis muscle of the right leg. A resting needle muscle biopsy was taken and analyzed for fiber type, SR function, and metabolite contents. A second biopsy was taken immediately after cessation of exercise and analyzed for muscle SR function and metabolite contents. Immediately after excision, the muscle was rapidly separated into portions, with one portion quickly frozen and stored in liquid nitrogen for subsequent metabolite determinations. The remaining portion was immediately weighed, homogenized, and frozen in liquid nitrogen for later SR function analyses. A portion of the resting biopsy sample was mounted using an embedding medium, quick-frozen in isopentane precooled in liquid nitrogen, and stored at −80°C until analysis of fiber types using the myofibrillar ATPase method (7). Fibers were classified into types I, IIa, or IIb according to their myofibrillar ATPase staining patterns after preincubation at pH 4.3, 4.6, and 10.3, and fiber type proportions were determined on the basis of the number of fibers in each classification.

Muscle SR Function

Skeletal muscle SR function was investigated in crude muscle homogenates as detailed previously (6, 45). Approximately 30–40 mg of muscle were weighed, diluted 1:10 (wt/vol) in a cold buffer containing Tris-HCl (40 mM, pH 7.9), sucrose (0.3 M), L-histidine (10 μM), EDTA (10 mM), and sodium azide (10 mM) and then homogenized on ice at 20,000 rpm for 3 × 15 s (Omni 1000, Omni International, Vernon, VA). The homogenate was then rapidly frozen in liquid nitrogen for later analyses of SR Ca2+ release, Ca2+-uptake, and Ca2+-ATPase activity.

Muscle Ca2+-uptake/release assay. The Ca2+ uptake and release rates were measured in duplicate in a standard buffer containing HEPES (20 mM, pH 7.0), KCl (150 mM), Mg-ATP (4.5 mM), indocarbocyanine (1 μM, Calbiochem), oxalate (7.5 mM), EDTA (10 mM), and Na2-ATPase-specific antibody (5 μM). The extravesicular starting free [Ca2+]i was determined in the absence of muscle homogenate by using the indo 1 ratiometric data and was calculated by using standard equations (24). This value varied between 0.8 and 1.0 μM but did not differ significantly between groups or rest vs. exercise samples. Slight modifications to previously described methods (45) include a higher oxalate concentration to increase vesicle Ca2+ loading and deletion of dithiothreitol from the homogenate buffer to avoid any inhibition of SR Ca2+-release. The buffer was stirred and maintained at 37°C. All measurements were completed within 50 min after thawing of the sample. The maximal rates of Ca2+-uptake into and release from vesicles formed by homogenization were monitored using indo 1. The reaction was initiated by the addition of 30–50 μl of homogenate to the buffer. Ca2+-uptake was mediated via Ca2+-ATPase activity because we have previously shown uptake to be inhibited by the addition of the specific inhibitor cyclopiazonic acid (CPA) to this assay medium (45), consistent with the findings of others (53). The Ca2+-uptake reaction was then allowed to stabilize before Ca2+ release was initiated by the addition of AgNO3 (141 μM) to the reaction buffer. It is known that Ag+ initiates Ca2+ release by oxidizing sulfhydryl groups on the RyR and that this Ca2+ release can be inhibited by the addition of the reducing agent dithiothreitol (45, 46, 53, 56). Although high concentrations of Ag+, such as used in this study, are known to depress SR Ca2+-ATPase activity in addition to inducing Ca2+ release, these effects are independent (8, 43). Furthermore, Ca2+-ATPase activity inhibition by Ag+ is beneficial through minimizing confounding Ca2+ release during the release measurement. Finally, separate experiments with and without CPA yielded no difference in the measured rate of Ag+-induced Ca2+ release, confirming that the release was not simply due to inactivation of Ca2+ pumps. In human muscle homogenates (n = 15 paired observations), the Ag+-induced Ca2+ release was not significantly different in the absence vs. presence of CPA (2.20 ± 0.38 vs. 1.83 ± 0.29 μmol·g−1·min−1, respectively). Preliminary experiments with the RyR modulators ruthenium red and caffeine were unsuccessful because of marked effects observed on the indo 1 ratio in these experimental conditions (data not shown), and these were therefore not employed. Spectral changes of indo 1 were monitored by using a luminescence spectrometer (AB2, SLM-Aminco, Urbana, IL). The sample was excited by a xenon lamp at 349 nm with a band pass of 1 nm; emission was measured at 410 nm for Ca2+-bound and at 485 nm for Ca2+-free forms of dye, with 8-nm band passes. A 410-to-485 nm fluorescence ratio was collected every 1 s. Minimum and maximum ratios were determined at the completion of the assay by the addition of EGTA (3.5 mM) and CaCl2 (5 mM), respectively. The dissociation constant for indo 1 was determined to be 164 nM, with the free [Ca2+]i calculated with the use of standard equations (24).

Muscle SR Ca2+-ATPase activity. The SR Ca2+-ATPase activity was determined at 37°C in triplicate, using 50 μl of homogenate and a spectrophotometric method (47). The total ATPase activity was first measured after the addition of 10 μl of 100 mM CaCl2, giving a final total concentration of 0.6 mM CaCl2 with a free [Ca2+]i of ~10 μM (47). The total ATPase activity (Mg2+-ATPase) was then measured after the addition of 20 μl of 2 mM CaCl2, giving a final concentration of 40 mM CaCl2, which selectively inhibits the SR Ca2+-ATPase. The SR Ca2+-ATPase activity was then determined from the total minus basal activities. The method is specific for SR Ca2+-ATPase activity, as indicated by its inhibition by both CPA (45) and a Ca2+-ATPase-specific antibody (47), by the close correspondence between Ca2+-ATPase activity measures in a homogenate preparation and purified SR, as well as the similarity [Ca2+]i and temperature dependence (47). The Ca2+ uptake and Ca2+-ATPase activity data are not used to determine turnover rates because of the markedly different free [Ca2+]i in the two assays, of ~1 and ~10 μM, respectively.

Muscle protein and calculations. SR measurements were expressed relative to muscle wet weight (μmol·min−1·g muscle−1) and, to identify the effects of any fluid shifts, muscle
protein content (μmol·min⁻¹·g protein⁻¹). Muscle protein was determined spectrophotometrically in triplicate, with albumin as a standard. The decline in SR function with fatigue is denoted by Δ (e.g., ΔCa²⁺ release) and the percent decline from rest values by %Δ (e.g., %ΔCa²⁺ release).

Variability of SR function measurements. We previously found no difference between repeat biopsies for SR Ca²⁺ uptake or Ca²⁺-ATPase activity in two subjects (6). To determine intrasubject variability, a vastus lateralis muscle biopsy was taken from separate incisions in the same leg in three control subjects (36.3 kg; means ± SE; n = 8). RT > ET (P < 0.05) and UT (P = 0.05).

RESULTS

Significant differences were found between groups for VO₂peak, sum of skinfolds, and FI during repeated maximal contractions (P < 0.05), with higher VO₂peak and lower sum of skinfolds, and FI in ET than in both UT and RT (Table 1). A significant main effect for training status was found for peak torque during dynamic isokinetic contractions (Fig. 1) being higher in RT than both ET (P < 0.05) and UT (P = 0.05). No significant group differences were found between groups for thigh circumference (Table 1). The FI results for the noninvasive trial on the Biodex dynamometer (48.6 ± 10.7, 50.1 ± 9.3, 31.2 ± 14.2% for UT, RT, and ET, respectively; means ± SD) did not differ from those in the invasive trial on the Cybex dynamometer (Table 1).

Muscle Fiber Type and Metabolites

Significant differences were found among groups for the type I and type IIa (P < 0.05) but not IIb (P = 0.13) fiber proportions (Table 2). ET had a higher proportion of type I fibers than UT and RT and a lower proportion of type IIa fibers than RT (P < 0.05, Table 2).

A significant exercise main effect (P < 0.05) was seen for each metabolite except ADP, with decreases in ATP, PCr, glycolyten, and pH and increases in lactate, Cr, and IMP (Table 3). A significant train-by-exercise interaction was found for PCr and Cr (P < 0.05). Resting muscle PCr was higher in RT than in UT (P < 0.05), which was higher than in ET (P < 0.05). After exercise, ET had higher PCr and lower Cr than RT and UT (P < 0.05).

Muscle SR Characteristics

Exercise and training comparisons. A significant exercise main effect was found for all SR variables, each
of which was depressed with fatigue (P < 0.05), whether expressed per muscle mass (µmol·min⁻¹·g muscle⁻¹) or muscle protein content (µmol·min⁻¹·g protein⁻¹).

**SR Ca²⁺ Release.** A significant exercise-by-train interaction was found for SR Ca²⁺ release (µmol·min⁻¹·g muscle⁻¹). Post hoc tests revealed that, in resting muscle, ET had lower maximal rates than UT and RT (21 and 17%, respectively, P < 0.05, Fig. 2) and that, after exercise, SR Ca²⁺ release declined (P < 0.05) to a similar level in all groups (Fig. 2). Similar results were found for SR Ca²⁺ release (µmol·min⁻¹·g protein⁻¹; Table 4).

The absolute (Δ) and relative (Δ%) declines in SR Ca²⁺ release with fatigue did not differ significantly between groups (Δ, P < 0.09, 0.83 ± 0.09, 0.91 ± 0.10, and 0.56 ± 0.13 µmol·min⁻¹·g muscle⁻¹; %Δ, P = 0.16, 42.1 ± 3.8, 43.4 ± 3.9, and 31.3 ± 6.1%; UT, RT, and ET, respectively). Similar findings were evident for declines in SR Ca²⁺ release expressed per gram protein (Δ, P = 0.12, %Δ, P = 0.34, Table 4).

**SR Ca²⁺ Uptake.** A significant exercise-by-train interaction was found for SR Ca²⁺ uptake (µmol·min⁻¹·g muscle⁻¹). Post hoc tests revealed that, in resting muscle, ET had lower maximal rates than UT (16%, P < 0.05, Fig. 3) and that SR Ca²⁺ uptake declined after exercise (P < 0.05) to a similar level in all groups (Fig. 3). Similar results were found for SR Ca²⁺ uptake per gram protein, with an additional observation that resting Ca²⁺ uptake in RT was 16% lower than in UT (P < 0.05, Table 4).

The ΔCa²⁺ uptake (µmol·min⁻¹·g muscle⁻¹) differed significantly between groups, being less (P < 0.05) in ET than in UT (0.81 ± 0.13, 0.56 ± 0.09, 0.43 ± 0.05; UT, RT, and ET, respectively), whereas the %ΔCa²⁺ uptake did not differ significantly between groups (P < 0.08, 43.0 ± 5.2, 34.1 ± 4.6, and 28.4 ± 2.8%; UT, RT, and ET, respectively). Similarly, the ΔCa²⁺ uptake (µmol·min⁻¹·g protein⁻¹) differed significantly between groups, being less in both ET and RT than in UT (P < 0.05), whereas the corresponding %ΔCa²⁺ uptake did not differ significantly between groups (P = 0.07, Table 4).

**SR Ca²⁺-ATPase Activity.** A significant exercise-by-train interaction was found for SR Ca²⁺-ATPase activity (µmol·min⁻¹·g muscle⁻¹). Post hoc tests revealed that, in resting muscle, ET had lower maximal rates than both RT and UT (22 and 23%, respectively, P < 0.05, Fig. 4) and that SR Ca²⁺-ATPase activity declined after exercise (P < 0.05) to a similar level in all groups (Fig. 4). Similar results were found for SR Ca²⁺-ATPase activity expressed per gram protein (P < 0.05, Table 4).

Both the ΔCa²⁺-ATPase activity and the %ΔCa²⁺-ATPase activity differed significantly between groups, each being less (P < 0.05) in ET than in RT (5.86 ± 0.82, 7.15 ± 0.83, and 3.74 ± 0.88 µmol·min⁻¹·g muscle⁻¹; and 38.6 ± 4.2, 48.5 ± 5.7, and 29.6 ± 5.0%, for UT, RT, and ET, respectively).

The ΔCa²⁺-ATPase activity (µmol·min⁻¹·g protein⁻¹) differed significantly between groups, being less in ET than in both RT and UT (P < 0.05, Table 4). The %ΔCa²⁺-ATPase activity did not differ significantly between the groups (P = 0.07, Table 4).

Variability of SR function measurements. Very similar SR Ca²⁺ uptake and Ca²⁺ release rates were found in measurements from two separate resting biopsies in the same individual (n = 3). The SR Ca²⁺ uptake was
1.01 ± 0.15 vs. 0.97 ± 0.15 μmol·g⁻¹·min⁻¹, whereas the Ca²⁺ release was 2.96 ± 1.19 vs. 3.32 ± 0.88 μmol·g⁻¹·min⁻¹, in resting biopsy 1 and in biopsy 2, respectively. When the same muscle sample in one individual was measured on separate days, almost identical SR Ca²⁺ release, Ca²⁺ uptake, and Ca²⁺-ATPase activity for both rest and fatigue measures were found. The values (day 1 rest, fatigue; day 2 rest, fatigue; μmol·g⁻¹·min⁻¹) for Ca²⁺ release were 1.35, 0.51; 1.35, 0.54; for Ca²⁺ uptake 1.38, 0.84; 1.50, 0.78; and for Ca²⁺-ATPase 10.65, 8.10; 10.74, 8.22.

**Relationships Between SR Function and Fiber Type**

Resting muscle. The SR Ca²⁺ release, Ca²⁺ uptake, and Ca²⁺-ATPase activity rates in resting muscle were each related to the type II fiber proportion (n = 24, P < 0.01, Fig. 5). SR function values (μmol·min⁻¹·g muscle⁻¹) for type I and II fibers were estimated by extrapolation from regression equations related to the type II fiber proportion by substituting values of 0 and 100 for type I and type II fiber values, respectively. This yielded estimates of 1.13 vs. 2.54 for SR Ca²⁺ release, 1.20 vs. 2.26 for Ca²⁺ uptake, and 8.61 vs. 20.71 for Ca²⁺-ATPase activity, respectively. The rates of SR Ca²⁺ uptake and Ca²⁺-ATPase activity were highly correlated (e.g., rest plus fatigue data pooled, r = 0.81, n = 48, P < 0.0001).

Fatigued muscle. The ΔCa²⁺ release (r = 0.58, P < 0.05), ΔCa²⁺ uptake (r = 0.40, P = 0.05), and ΔCa²⁺-ATPase activity (r = 0.43, P < 0.05) pooled for all subjects were also related to the type II fiber proportion (n = 24, μmol·min⁻¹·g muscle⁻¹, Fig. 6). The ΔCa²⁺ release was related to both ΔCa²⁺ uptake (r = 0.53, P < 0.01) and ΔCa²⁺-ATPase activity (r = 0.65, P < 0.001) with the latter two also related (r = 0.47, P < 0.05).

**Relationships Between SR Function and Metabolites**

To examine possible relationships between muscle metabolites and SR function, the rest and fatigue data were pooled for all subjects (n = 44). Muscle Ca²⁺

---

**Table 4. Effects of fatigue on SR Ca²⁺ release, uptake and Ca²⁺-ATPase activity in UT, RT, and ET human skeletal muscle, expressed relative to muscle protein**

<table>
<thead>
<tr>
<th>SR Variables</th>
<th>UT Rest</th>
<th>Fatigue</th>
<th>RT Rest</th>
<th>Fatigue</th>
<th>ET Rest</th>
<th>Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺ release</td>
<td>12.9 ± 1.0</td>
<td>7.4 ± 0.6a</td>
<td>12.7 ± 0.8</td>
<td>7.3 ± 0.8a</td>
<td>10.1 ± 0.6†‡</td>
<td>6.7 ± 0.3a</td>
</tr>
<tr>
<td>Δ g protein⁻¹</td>
<td>5.5 ± 1.0</td>
<td>41.0 ± 6.3</td>
<td>5.4 ± 0.7</td>
<td>42.2 ± 4.3</td>
<td>3.4 ± 0.6</td>
<td>31.9 ± 5.0</td>
</tr>
<tr>
<td>Ca²⁺ uptake</td>
<td>11.9 ± 0.8</td>
<td>6.2 ± 0.4a</td>
<td>10.0 ± 0.4†</td>
<td>6.6 ± 0.5a</td>
<td>9.4 ± 0.2†</td>
<td>6.6 ± 0.2a</td>
</tr>
<tr>
<td>Δ (%)</td>
<td>5.7 ± 1.0</td>
<td>45.7 ± 6.6</td>
<td>3.4 ± 0.6†</td>
<td>33.2 ± 4.7</td>
<td>2.8 ± 0.3†</td>
<td>29.3 ± 2.6</td>
</tr>
<tr>
<td>Ca²⁺-ATPase</td>
<td>96.1 ± 2.9</td>
<td>56.0 ± 4.7a</td>
<td>90.4 ± 4.9</td>
<td>48.1 ± 7.1*</td>
<td>71.9 ± 2.4†‡</td>
<td>50.6 ± 3.0a</td>
</tr>
<tr>
<td>Δ</td>
<td>40.1 ± 4.8</td>
<td>41.7 ± 4.5</td>
<td>42.3 ± 5.5</td>
<td>47.6 ± 6.5</td>
<td>21.3 ± 4.3†‡</td>
<td>28.9 ± 5.1</td>
</tr>
<tr>
<td>Δ (%)</td>
<td>41.7 ± 4.5</td>
<td>41.7 ± 4.5</td>
<td>41.7 ± 4.5</td>
<td>41.7 ± 4.5</td>
<td>41.7 ± 4.5</td>
<td>41.7 ± 4.5</td>
</tr>
</tbody>
</table>

Values are means ± SE (in μmol·min⁻¹·g protein⁻¹). Δ and Δ (%), Absolute decline and percent decline, respectively, in sarcoplasmic reticulum (SR) function from rest. *Fatigue < rest within group; † < UT corresponding sample; and ‡ < RT corresponding sample (all P < 0.05).
SR Ca\(^{2+}\) Regulation and Fatigue in Humans

**Fig. 5. Relationships between the maximal rates of SR Ca\(^{2+}\) release (A), Ca\(^{2+}\) uptake (B), and Ca\(^{2+}\)-ATPase activity (C) measured in resting muscle and the proportion of type II muscle fibers. SR units are \(\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g muscle}^{-1}\); \(n = 24\). ○, UT; ▽, RT; and □, ET. Regression lines are for pooled data and equations are as follows: Ca\(^{2+}\) release \((0.018 \times \text{type II}%) + 1.134 (r = 0.62, P < 0.005)\); Ca\(^{2+}\) uptake \((0.011 \times \text{type II}%) + 1.199 (r = 0.52, P < 0.01)\); Ca\(^{2+}\)-ATPase \((0.121 \times \text{type II}%) + 8.606 (r = 0.56, P < 0.005)\).**

Release \((0.51 \leq r \leq 0.77, P < 0.001)\), Ca\(^{2+}\) uptake \((0.49 \leq r \leq 0.76, P < 0.001)\) and Ca\(^{2+}\)-ATPase activity \((0.46 \leq r \leq 0.74, P < 0.001)\) were each related to muscle ATP, PCr, glycogen, and pH. Inverse relationships were found between muscle Ca\(^{2+}\) release \((-0.53 \leq r \leq -0.72, P < 0.001)\), Ca\(^{2+}\) uptake \((-0.55 \leq r \leq -0.76, P < 0.001)\), and Ca\(^{2+}\)-ATPase activity \((-0.42 \leq r \leq -0.66, P < 0.001)\) against muscle lactate, IMP, and Cr contents. These significant relationships could suggest a direct link between muscle metabolites and in vitro SR function. However, correlations between ∆SR and ∆metabolite measures with fatigue were inconsistent (data not shown), suggesting the magnitudes of metabolic disturbance and of SR dysfunction measured in vitro were not directly related.

**Relationships Between SR Function, Fiber Type, and Performance**

The FI for all subjects \((n = 24\), Fig. 7) was related to the ∆Ca\(^{2+}\) release, ∆Ca\(^{2+}\) uptake (both \(r = 0.51, P < 0.05\)), and ∆Ca\(^{2+}\)-ATPase activity \((r = 0.52, P < 0.01)\), as well as to each of the %∆SR variables \((0.43 < r < 0.53, P < 0.05)\). The FI was related \((r = 0.57, P < 0.005)\) and \(\dot{V}O_2\) peak inversely related \((r = -0.62, P < 0.005)\) to the proportion of type II fibers.

**DISCUSSION**

This study reports several unique findings on the effects of intense fatiguing contractions on skeletal muscle SR function, measured under standardized in vitro conditions, in each of UT, ET, and RT subjects. We show for the first time that SR Ca\(^{2+}\) release in human muscle is markedly depressed with fatigue in UT subjects as well as in athletes and that the depression was significantly related to the extent of muscle fatigability. These findings indicate that impaired SR function is an obligatory response to fatiguing contractions and point toward a key involvement of SR dysfunction in muscular fatigue in humans. Both fiber type and training appear to influence SR function in resting muscle. Lower SR Ag\(^{+-}\)-induced Ca\(^{2+}\) release and SR Ca\(^{2+}\) uptake rates were found in resting muscle in ET, consistent with their low type II fiber proportion; however, fiber type differences could not explain the lower muscle Ca\(^{2+}\) uptake found in RT than controls.
SR Ca\(^{2+}\) REGULATION AND FATIGUE IN HUMANS

Fatigue Depresses Ag\(^+\)-induced SR Ca\(^{2+}\) Release and SR Ca\(^{2+}\)-ATPase Activity in Human Muscle

Fatiguing voluntary contractions reduced both muscle peak torque and maximal Ag\(^+\)-induced SR Ca\(^{2+}\) release rate by ~42% in untrained subjects. The reduced SR Ca\(^{2+}\) release rate in untrained human muscle is consistent with reductions with fatigue found in other species, utilizing similar methodology (15, 53, 56) and in humans of unspecified training status (27). Our findings are also consistent with the decline in fatigue with the rapidly releasable Ca\(^{2+}\) in stimulated intact single fibers (30), as well as with SR Ca\(^{2+}\) release elicited by caffeine in skinned single frog fibers (55).

Ag\(^+\) induces Ca\(^{2+}\) release via oxidizing SH groups on the physiological release channel, the RyR, and this can be inhibited by SH reducing agents such as dithiothreitol (26, 45, 46, 53, 56). Although Ag\(^-\)-induced Ca\(^{2+}\) release is specific for Ca\(^{2+}\) release from the RyR (14), several limitations exist in our methodology. First, neither Ag\(^-\) nor the process of RyR oxidation is involved in the physiological induction of SR Ca\(^{2+}\) release (34), and thus the physiological significance of our findings remains to be elucidated by further study. Caffeine is commonly used to study fatigue effects on SR Ca\(^{2+}\) release because of its specific effects on the RyR (14). However, caffeine could not be utilized here because of its marked effects on indo 1 at the high concentrations required to induce release in human muscle. Importantly, however, others have reported qualitatively similar responses to fatigue, or to conditions mimicking fatigue, in Ca\(^{2+}\) release induced by Ag\(^+\), caffeine, and 4-chloro-m-cresol (14, 53, 56). In particular, Ag\(^+\) and 4-chloro-m-cresol-mediated Ca\(^{2+}\) release rates were similarly depressed under conditions of graded fatigue (53). Hence, it is highly probable that our findings of reduced Ag\(^-\)-induced Ca\(^{2+}\) release are valid indicators of depressed SR release in human muscle. Finally, the measured Ag\(^+\)-induced Ca\(^{2+}\) release rate will be affected by Ca\(^{2+}\) buffering due to oxalate, EDTA, and muscle proteins within the assay medium and homogenate. Therefore, the measured Ca\(^{2+}\) release (and Ca\(^{2+}\) uptake) rates reported here should be compared only with other measures using the same experimental conditions. Because all assay conditions were constant, the comparisons between rest and exercise and between groups are valid.

We report for the first time a decline in Ca\(^{2+}\)-ATPase activity in human muscle after maximal contractions and confirm proportional and significantly correlated reductions in SR Ca\(^{2+}\) uptake (43%) and Ca\(^{2+}\)-ATPase activity (39%). Thus neither maximal contractions, nor prolonged submaximal contractions (6, 52) appear to differentially affect the hydrolytic and Ca\(^{2+}\) vectorial transport properties of the Ca\(^{2+}\)-ATPase enzyme. Thus the recent report of reduced SR Ca\(^{2+}\) uptake but unchanged Ca\(^{2+}\)-ATPase activity with fatigue (27) is inconsistent with our findings and those of other human studies. The large percent reduction in muscle SR Ca\(^{2+}\) uptake after intense contractions in the present study was similar to other reports in humans (20, 25) and in horses (10) but twice that found in humans after prolonged exercise (6). This effect most likely reflects the greater muscular fatigue and thus depression in SR function induced by intense contractions (53).

An important finding was that in vitro SR Ca\(^{2+}\) release, Ca\(^{2+}\) uptake, and Ca\(^{2+}\)-ATPase activity were significantly depressed with fatigue in both ET and RT groups, as well as in the UT group. Thus chronic training did not prevent the decline in SR function with fatigue. Neither the absolute (Δ) nor the relative (%Δ) decline in SR Ca\(^{2+}\) release differed between groups and both variables were correlated with the FI. This suggests that depressed Ca\(^{2+}\) release is an essential component of muscle fatigue during voluntary exercise in humans. The relationship between the FI and all SR variables is also consistent with an earlier finding in rat muscle that the extent of depression in SR function is dependent on the severity of fatigue (53). The relative decline with fatigue in SR Ca\(^{2+}\) release was ~9% less (not significant) in the ET than in UT, consistent with the lesser decline in SR function in subjects with a predominance of type I fibers. The lack of significance of a training effect most likely reflects the modest statistical power of the study. Together with the cross-sectional design of the study, these sug-

Fig. 7. Relationships between the fatigue index (FI) and the fatigue-induced absolute decline in the maximal rates of Ca\(^{2+}\) release (A), Ca\(^{2+}\) uptake (B), and Ca\(^{2+}\)-ATPase activity (C) in vastus lateralis muscle during the 50 repeated maximal quadriceps contractions. SR units are μmol·min\(^{-1}\)·g muscle\(^{-1}\); n = 24. ●, UT; ▽, RT; and □, ET. Regression lines are for pooled data and equations are as follows: FI (%) = (21.41 × ΔCa\(^{2+}\) release) + 23.8 (r = 0.51, P < 0.05); FI (%) = (22.56 × ΔCa\(^{2+}\) uptake) + 26.61 (r = 0.51, P < 0.05); FI (%) = (2.63 × ΔCa\(^{2+}\)-ATPase) + 25.51 (r = 0.52, P < 0.01).
gest a likely type II error with respect to training effects on the decline in SR Ca^{2+} release with fatiguing exercise. The ΔCa^{2+} uptake and ΔCa^{2+}-ATPase activity (per g protein) were less in ET than in UT, consistent with their lower proportion of type II fibers. Overall, our RT data suggest that resistance training does not attenuate the ΔSR function during repeated maximal contractions.

Role of Fiber Type and Metabolism in Depressed SR Function with Fatigue

The significant relationships between depressed SR function with intense contractions and type II fiber proportion strongly suggest a greater susceptibility of SR to fatigue in type II fibers in humans. This conclusion must, however, remain equivocal because SR function was measured in crude homogenates of whole muscle, not in single fibers, but is consistent with findings in other species and with the greater fatigability in type II fibers (48). We cannot discern the actual mechanisms underlying reductions in SR Ca^{2+} release and Ca^{2+}-ATPase activity with fatigue. However, SR function was measured in vitro under standardized conditions, which implicates structural alterations in the RyR and Ca^{2+}-ATPase proteins, consistent with other studies using vesicles (6, 20, 25, 40, 56) or single-fiber models (55). Potential mechanisms responsible for in vivo failure of SR Ca^{2+} release include metabolic and ionic disturbances such as P_i, Mg^{2+}, and Ca^{2+} accumulation and localized depletion of ATP, PCR, and glycogen (3, 13, 14, 18, 30, 42). Our findings strongly suggest that metabolic disturbances do not directly induce structural alterations in SR in human muscle. Rather, the depressive effects of local metabolic changes on Ca^{2+} release reported by others, together with the structural alterations that we find in vitro, indicate that an even greater in vivo depression in SR Ca^{2+} release must occur in severe muscle fatigue. Additional inhibitory factors for Ca^{2+} release might include cytosolic Ca^{2+} accumulation linked with reduced SR Ca^{2+} uptake (6, 13, 35, 54, 55), decline in glycogen (11, 48), and increased reactive oxygen species (33). Thus a large reduction in SR Ca^{2+} release is likely to be a major contributor to depressed muscle force with fatigue (1, 30). Interestingly, the depression in SR Ca^{2+} release was significantly related to SR Ca^{2+}-ATPase, which may reflect common mechanisms or perhaps a coordinated downregulation of the two Ca^{2+} regulatory proteins.

Muscle Fiber Composition and Training Affect SR Function in Resting Muscle

A new finding in human muscle was the relationship between SR Ca^{2+} release and fiber composition, with an approximately twofold higher Ca^{2+} release rate estimated in type II than type I fibers. The Ca^{2+} uptake rate and Ca^{2+}-ATPase activity were also estimated to be approximately two- to threefold higher in type II than type I fibers. The Ca^{2+}-ATPase activity results are consistent with other studies showing Ca^{2+}-ATPase dependence on fiber type in human muscle (2, 38, 50). These findings most likely reflect a higher RyR and Ca^{2+}-ATPase density in type II than in type I fibers, as reported in other species (e.g., Ref. 44). Different expression of RyR and Ca^{2+}-ATPase isoforms are unlikely to explain these estimated fiber type differences because only the RyR1 isoform is expressed in muscle, whereas activity differences in the slow and fast Ca^{2+}-ATPase isoforms are minor.

Muscle SR characteristics clearly varied between the different training groups. However, the lower SR Ca^{2+} release, Ca^{2+} uptake, and Ca^{2+}-ATPase activity in ET appeared to be entirely consistent with their lower proportion of type II fibers. The cross-sectional experimental design employed makes it impossible to discern whether endurance training exerts any additional effects on muscle SR characteristics beyond those differences due to muscle fiber type, but these training effects are likely to be small in humans (32, 38). Specific training effects on muscle SR are suggested in RT, in which SR Ca^{2+} uptake was significantly lower than in controls, without differences in fiber composition. This is consistent with decreased Ca^{2+}-ATPase protein expression, Ca^{2+} uptake, and Ca^{2+}-ATPase activity in rat fast-twitch muscle after muscle loading (31) but differs from the unchanged resting Ca^{2+}-ATPase activity or content with short-term and chronic resistance training in young and elderly humans (23, 32). The reasons for this are unclear. The similar SR Ca^{2+} release in RT and UT may indicate that RyR are upregulated in proportion to muscle mass. Interestingly, decreased muscle RyR content was found in long-term RT elderly men (32), although this does not necessarily imply that maximal Ca^{2+} release rates would also be lower. On the other hand, both RyR expression and SR Ca^{2+} release were increased in resting muscle after sprint training, without change in fiber composition (40). The lack of effect of RT on Ca^{2+} release might reflect the fact that our RT group did not appear to be as highly trained as the ET group, although their RT training status was confirmed by greater dynamic torque production. Overall, our findings therefore suggest that chronic endurance and resistance training do not induce major changes in SR functional characteristics but, rather, that these are dominated by the muscle fiber composition.

Contractile and Performance Implications

The changes in intracellular [Ca^{2+}] during contractions in healthy human muscle fibers are unknown. However, it is likely that an ~40% reduction in SR Ca^{2+} release with fatigue in human muscle would significantly reduce intracellular [Ca^{2+}] and force production. For force-pCa relationships in human vastus lateralis fibers (36), a 40% decline in intracellular [Ca^{2+}] from physiological levels (i.e., 1 μM) would reduce force by 25, 36, and 45% in type I, IIa, and IIb fibers, respectively. The greater decrease in SR Ca^{2+} release in those subjects with a high proportion of type II fibers and the correlations between both variables...
and the muscle FI provide further evidence of the important role of depressed Ca\(^{2+}\) release in fatigue in human muscles. Decreased SR Ca\(^{2+}\) uptake would initially attenuate the decline in intracellular [Ca\(^{2+}\)] and force with fatigue but may eventually also lead to inadequate SR loading, thus reducing the Ca\(^{2+}\) store available for rapid release, and exacerbate fatigue. It therefore seems reasonable to conclude that impaired SR Ca\(^{2+}\) release and Ca\(^{2+}\) uptake are important factors in the reduced muscle force with fatigue in humans. However, reduced in vitro SR function clearly cannot fully explain the reduction in muscle torque with fatigue, because the ΔSR function variables accounted for only ~26% of the variance in the FI. This is not unexpected, because the in vivo depression in SR function is probably larger than our in vitro measures, but also indicates the importance of additional non-SR factors in muscle fatigue. These may include involvement of central factors, as well as within muscle, each of a rundown of cellular sodium and potassium gradient effects affecting T-tubular excitability, decreased myofibrillar Ca\(^{2+}\) sensitivity, and depressive effects of P\(_i\) accumulation on the contractile proteins (17).

In conclusion, this study advances our understanding of fatigue, training, and muscle fiber composition effects on SR function in human muscle. The maximal in vitro SR Ca\(^{2+}\) release rate was markedly reduced after voluntary fatiguing contractions, with proportional depressions in in vitro Ca\(^{2+}\) uptake and Ca\(^{2+}\)-ATPase activity. These findings indicate that SR function in human muscles is similarly affected by fatigue as in other species. Human SR function is clearly dependent on muscle fiber type but may be additionally influenced by training status. ET athletes had lower SR function than controls, although this was consistent with their lower proportion of type II fibers. RT athletes had an unchanged fiber composition and thus similar SR Ca\(^{2+}\) release to controls, but lower SR Ca\(^{2+}\) uptake, which suggests additional resistance training effects. Finally, chronic training did not prevent or even significantly attenuate the depressive effects of fatiguing exercise on muscle SR Ca\(^{2+}\) release. This points to a possible causal link between SR dysfunction and fatigue in contracting human muscle.

We thank our subjects for generosity and hard work; Dr. Andrew Garnham and Dr. Peter Braun for performing the muscle biopsies; Dr. Ternmoob Sangkabutra, Dr. Steve Selig, Dr. Judy Morton, Simon Sostarine, and Jim Leppik for assistance in the experiments; and Dr. Rod Snow for critical comments on the manuscript.

This study was supported in part by the Australian Research Council.

REFERENCES


43. Prabhu SD and Salama G. The heavy metals Ag + and Hg 2+ trigger calcium release from cardiac sarcoplasmic reticulum. Arch Biochem Biophys 15: 47–55, 1990.


