Adaptations in human muscle sarcoplasmic reticulum to prolonged submaximal training

H. J. Green,1 C. S. Ballantyne,2 J. D. MacDougall,2 M. A. Tarnopolsky,3 and J. D. Schertzer1
1Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1; and
2Departments of Kinesiology and Medicine, McMaster University, Hamilton, Ontario, Canada L8S 4K7
Submitted 22 March 2002; accepted in final form 22 January 2003

Green, H. J., C. S. Ballantyne, J. D. MacDougall, M. A. Tarnopolsky, and J. D. Schertzer. Adaptations in human muscle sarcoplasmic reticulum to prolonged submaximal training. J Appl Physiol 94: 2034–2042, 2003; 10.1152/japplphysiol.00244.2002.—In this study, we employed single-leg submaximal cycle training, conducted over a 10-wk period, to investigate adaptations in sarcoplasmic reticulum (SR) Ca2+-regulatory proteins and processes of the vastus lateralis. During the final weeks, the untrained volunteers (age 21.4 ± 0.3 yr; means ± SE, n = 10) were exercising 5 times/wk and for 60 min/session. Analyses were performed on tissue extracted by needle biopsy ~4 days after the last training session. Compared with the control leg, the trained leg displayed a 19% reduction (P < 0.05) in homogenate maximal Ca2+-ATPase activity (192 ± 11 vs. 156 ± 18 μmol·g protein−1·min−1), a 4.3% increase (P < 0.05) in pCa50, defined as the Ca2+ concentration at half-maximal activity (6.01 ± 0.05 vs. 6.26 ± 0.07), and no change in the Hill coefficient (1.75 ± 0.15 vs. 1.76 ± 0.21). Western blot analysis using monoclonal antibodies (7E6 and A52) revealed a 13% lower (P < 0.05) sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) 1 in trained vs. control in the absence of differences in SERCA2a. Training also resulted in an 18% lower (P < 0.05) SR Ca2+ uptake and a 26% lower (P < 0.05) Ca2+ release. It is concluded that a downregulation in SR Ca2+ cycling in vastus lateralis occurs with aerobic-based training, which at least in the case of Ca2+ uptake can be explained by reduction in Ca2+-ATPase activity and SERCA1 protein levels.

REGULAR CONTRACTILE ACTIVITY is a potentially potent stimulus for altering the composition, structure, and function of the muscle cell. Nowhere is this more evident than with the chronic low-frequency electrical stimulation (CLFS) model in which contractile activity is characteristically induced for 12–24 h/day over several weeks. Induced patterns of submaximal contractions of this nature typically applied to muscles composed of a predominance of fast-twitch (type II) fibers result in extensive reorganization of both the excitation and contraction processes and the energy metabolic pathways involved in energy supply. Although the magnitude of the adaptations vary, qualitatively similar adaptations appear to occur between species (dog, rat, mouse, rabbit) (29).

Despite the fact that the CLFS model has proved invaluable for studying the limits of phenotypic plasticity in skeletal muscle and the factors regulating the expression of a large number of proteins involved in fiber-type transformation, it is nonphysiological (30). In physiological models, voluntary activity is used to elicit training adaptations. Voluntary training typically involves a relatively brief session of exercise followed by a prolonged recovery period, which could last for 2–3 days depending on the training frequency. Moreover, it is not clear how the intracellular signals mediating altered protein expression in CLFS translate into different programs of voluntary training. Unlike CLFS, the properties that have been explored with voluntary training have been much more limited. To a large extent, the focus has been directed toward the energy metabolic pathways and more recently the contractile protein myosin. The processes involved in excitation-contraction coupling, namely those involved in the transmission of the neural signal to the interior of the fiber, and which result in an increase in the free cytosolic Ca2+ ([Ca2+]i) in signal involved in myofibrillar activation and muscle contraction, has received scant attention.

One vital organelle that is the primary regulator of [Ca2+]i levels in the skeletal muscle cell is the sarcoplasmic reticulum (SR). The SR is a membranous network that envelops the myofibrils and acts as a storage depot for Ca2+. Also embedded in the SR is a collection of special proteins that regulate the release of Ca2+ into the cytosol and the sequestration of Ca2+ back into the lumen of the SR. The ry Rhodes receptor (RyR) or Ca2+-release channel and the SR Ca2+-ATPase (SERCA) are the principal proteins involved in Ca2+ release and Ca2+ uptake, respectively. Both RyR and SERCA exist as several isoforms in mammalian tissue (1, 8).

As with a variety of other cellular proteins, the proteins involved in SR Ca2+ cycling can also undergo extensive replacement when CLFS is administered to fast-twitch (type II)-based muscles. Provided that CLFS is of sufficient daily volume and duration, RyR...
protein content decreases dramatically (9, 14, 26) without changes in RyR1 to RyR2, the isoform that predominates in heart muscles (8). Although not measured, the downregulation in RyR would be expected to be accompanied by a pronounced decrease in Ca\(^{2+}\)-release kinetics. The CLFS model can also induce large reductions in SERCA content. However, unlike RyR, a shift from SERCA1, the predominant isoform observed in type II muscle fibers, to SERCA2a, the major isoform that exits in slow-twitch (type I) fibers, occurs (3, 14, 26, 27). Because the alteration in SERCA isoform content with CLFS might be expected to change, the Ca\(^{2+}\) affinity as measured by the Hill coefficient and Ca\(^{2+}\) concentration at half-maximal activity (pCa\(_{50}\)), it is surprising that no study has apparently addressed this issue (29). Similarly, the coupling ratio, defined as the ratio between Ca\(^{2+}\) uptake and Ca\(^{2+}\)-ATPase activity, appears unexplored with CLFS (3). On the basis of the adaptations that occur with CLFS, it is reasonable to suggest that the functional alterations in SR Ca\(^{2+}\) cycling occur, in large part, as a result of a reduction in protein content both to the RyR and SERCA.

In contrast to CLFS, SR adaptations to voluntary training remain poorly characterized, particularly with regard to the relationship between the nature of the contractile stimulus and the resulting alterations in protein expression and function. At least in terms of the contractile intensity and the strain put on the excitation-contraction processes and metabolic flux, low-intensity aerobic running would appear to have a close parallel to CLFS. As a consequence, the adaptations that occur with a training program of this nature would be expected to resemble, at least qualitatively, those occurring with CLFS. There is some evidence that this occurs. In one of the earliest studies using treadmill running in rats, Kim et al. (17) reported decreases in maximal SR Ca\(^{2+}\) uptake (V\(_{\text{max}}\)) and in Ca\(^{2+}\) affinity but only in tissue composed of a predominance of fast-twitch, low-oxidative fibers and not in tissue of predominant fast-twitch, high-oxidative fibers or slow-twitch-based muscle. That the reduction in V\(_{\text{max}}\) is due at least in part to a reduction in SR Ca\(^{2+}\)-ATPase protein is supported by the findings of Green et al. (12), who reported reductions in Ca\(^{2+}\)-ATPase protein content after a similar but more extreme running program also using rats.

Additional evidence that the adaptations in the SR may be specific to the nature of the training has been recently published using sprint training in humans (28). In this study, large increases in both SERCA1 and SERCA2 protein content were observed in tissue obtained from the vastus lateralis. Surprisingly, however, the increases in the Ca\(^{2+}\)-ATPase protein were not accompanied by increases in maximal Ca\(^{2+}\)-ATPase activity or in Ca\(^{2+}\)-uptake kinetics. These investigators also reported increases in SR Ca\(^{2+}\)-release rates that were accompanied by increases in RyR protein content as measured by Western blot but not by \(^{3}H\)ryanodine binding (28). These results suggest that modification of the protein levels of the SR may occur independently of changes in the functional properties of the SR. At present it is unclear whether this effect is species specific or due to the nature of the exercise used for training.

The purpose of this study was to investigate the alterations in the Ca\(^{2+}\)-exchange properties of the SR in human muscle in response to a training program involving prolonged aerobic-based exercise. We have hypothesized that, similar to CLFS, reductions in Ca\(^{2+}\) uptake and Ca\(^{2+}\)-ATPase would occur in conjunction with a shift toward SERCA2a, the predominant isoform of type I fibers, and that these adaptations would occur independently of changes in Ca\(^{2+}\) sensitivity or coupling ratios. Moreover, we have also postulated that the adaptations in Ca\(^{2+}\) sequestration would be accompanied by reductions in Ca\(^{2+}\)-release kinetics.

**METHODS**

**Subjects.** Ten healthy men (age 21.4 ± 0.3 yr; body mass 76.8 ± 2.2 kg; means ± SE) volunteered for the study. All participants were active but not engaged in exercise, either high resistance or endurance, on a regular basis for at least 6 mo before the beginning of the study. Written consent was obtained from all volunteers as required after approval of the study by the Office of Human Research.

**Experimental design.** The training program consisted of 10 wk of single-leg submaximal cycle performed in the recumbent position. Participants cycled at 60 revolutions/min at a power output that initially corresponded to 75% of the pretraining peak power output. Training initially consisted of 3 × 30 min sessions/wk, gradually progressing to 5 × 60 min sessions/wk. The power output was adjusted throughout the training to maintain individual target heart rates in the range of 140 and 160 beats/min. The leg assigned for training, dominant vs. nondominant, was randomly assigned. The dominant leg was selected on the basis of the leg preferred in a vertical pump with a single foot takeoff. For each participant, the other leg served as the untrained control.

Before the beginning of training, each volunteer performed single-leg progressive cycle tests for measurement of peak aerobic power (V\(_{\text{O}_2,\text{peak}}\)). Individual tests, separated by at least 24 h, were performed on each leg in randomized order. In the progressive cycle protocol, a brief warm-up was provided before application of square increases in power output every 2 min. The V\(_{\text{O}_2,\text{peak}}\), measured with open-circuit spirometry (23), was defined as the highest value averaged over 60 s that was attained before fatigue. The V\(_{\text{O}_2,\text{peak}}\) protocols were again measured 2 days after the training period.

Approximately 4 days after the last training session, tissue samples were extracted from the vastus lateralis muscle under local anaesthesia by using the needle biopsy technique with manual suction. Tissue was rapidly extracted from the control and trained legs and sectioned into different samples. One sample was rapidly frozen in liquid N\(_2\), and another sample was used to prepare an homogenate for eventual measurement of SR properties. All samples were stored at −80°C until analyses.

**Measurements of SR function.** Measurements of Ca\(^{2+}\)-ATPase, Ca\(^{2+}\) uptake, and Ca\(^{2+}\) release were performed on stored homogenates previously made from tissue samples prepared immediately after extraction from the vastus lateralis and stored at −80°C. Homogenates were prepared by use of 11:1 (vol/wt) dilution of buffer containing (in mM) 200 sucrose, 40 l-histidine, 1 EDTA, 10 Na\(_{2}\)Ac, and 1 DTT (pH 7.8) by using a hand-held glass homogenizer (Kontes, Duall...
20) (24). The homogenate was separated into a number of aliquots, quick-frozen in liquid N₂, and stored at −80°C. Immediately before analysis, aliquots were thawed and processed for specific measurements.

**Ca²⁺-ATPase activity.** Total Ca²⁺-ATPase activity was measured by using the turbidimetric assay developed by Simonides and van Hardeveld (37) with minor modifications. The reaction buffer contained (in mM) 200 KCl, 80 HEPES, 15 MgCl₂, 1 EGTA, 10 NaN₃, 5 ATP, and 10 phos-phenol-sulfuric acid (pH 7.0). The pH was adjusted at 37°C. Just before starting the reaction, 18 µM lactate dehydrogenase (LDH), 18 U/ml pyruvate kinase (PK), 33 µM enolase (31), 1 µM Ca²⁺ ionophore A-23187 (Sigma C-7522), and 0.3 mM NADH were added to a cuvette containing 1 ml of reaction buffer. Assays were performed in duplicate at 37°C and 340 nm (Shimadzu UV 160) with 1 mg wet wt of tissue per assay. After the recording of baseline absorbance and fluorescence of NADH for −1 min, the reaction was initiated by addition of 100 mM CaCl₂ and was monitored for 1–2 min. Maximal Ca²⁺-ATPase activity and Ca²⁺-dependency of Ca²⁺-ATPase activity were assessed by using CaCl₂ in 0.5 µM and CaCl₂ 1 µM. Ca²⁺-ATPase activity increases with [Ca²⁺]₀ until a plateau occurs, once maximal activity is reached. The [Ca²⁺]₀ corresponding to each CaCl₂ addition was assessed separately by using dual-wavelength spectrofluorometry and the Ca²⁺-fluorescent dye indo-1. The measurement of [Ca²⁺]₀ using this procedure is based on the difference in emission wavelengths between the Ca²⁺-bound-indo-1 complex and the Ca²⁺-free-indo-1 complex. An excitation wavelength of 365 nm was used, and the emission maxima were recorded at 405 and 485 nm for Ca²⁺-bound (G) and Ca²⁺-free (F) indo-1. The ratio of F to G decreases during Ca²⁺ uptake and was used to calculate [Ca²⁺]₀. Felix software (Photon Technology International) was used to calculate the ionized Ca²⁺ concentration by use of Eq. 2.5 of Grynkiewicz et al. (13)

\[
[\text{Ca}^{2+}]_0 = K_d \frac{G_{\text{max}}/G_{\text{min}}}{(R - R_{\text{min}})/(R_{\text{max}} - R)}
\]

where \(K_d\) is the equilibrium constant for the interaction between Ca²⁺ and indo-1, \(R_{\text{min}}\) is the minimum value of R with the addition of 250 µM EGTA, \(G_{\text{max}}\) is the maximum value of G with the addition of 250 µM EGTA, \(G_{\text{min}}\) is the minimum value of G with the addition of 1 mM CaCl₂, and \(R_{\text{max}}\) is the maximum value of R with the addition of 1 mM CaCl₂. Simultaneous photon counts per second were recorded for both emission wavelengths. Before the experiment, Ca²⁺-independent background fluorescence was recorded in the reaction medium in the absence of indo-1 and was used to correct each assay. The dissociation constant \(K_d\) that was used for the interaction between Ca²⁺ and indo-1 was 250 nM (13). It should be emphasized that in homogenates the actual \(K_d\) is significantly affected by the protein composition. Ca²⁺-ATPase activity was obtained by subtracting basal activity from total (Mg²⁺-activated) ATPase activity. Basal activity was measured in the presence of 40 µM cyclopiazonic acid, which completely inhibits SR Ca²⁺-ATPase activity (35). The validity of the SR Ca²⁺-ATPase assay using whole muscle homogenates has been previously demonstrated in both animals (37) and humans (31).

The kinetic properties of the Ca²⁺-ATPase that were assessed included the maximal activity \(V_{\text{max}}\), the \([\text{Ca}^{2+}]_0\) needed to obtain half-maximal activity, and the Hill coefficient. To obtain the kinetic properties of the enzyme, Ca²⁺-ATPase activity was plotted against the negative logarithm of \([\text{Ca}^{2+}]_0\) (pCa). The \(V_{\text{max}}\) represented the peak value, whereas the \(pC_{\text{Rmax}}\) represented the pCa obtained from a sigmoid fit of the data, which yields 50% of \(V_{\text{max}}\). The Hill coefficient was determined through a nonlinear regression with computer software (Graph Pat Software) by using a portion of the curve that corresponded to between 20 and 80% of maximal activity, using the following sigmoidal dose-response equation

\[
Y = Y_{\text{bot}} + \frac{Y_{\text{top}} - Y_{\text{bot}}}{1 + 10^{(p\text{Ca} - 7)}}
\]

where \(Y_{\text{bot}}\) is the value at the bottom of the plateau (20%) and \(Y_{\text{top}}\) is the value at the top (80%), \(p\text{Ca}\) is the concentration that gives a response halfway between \(Y_{\text{bot}}\) and \(Y_{\text{top}}\), and \(H\) is the Hill coefficient.

**Ca²⁺ uptake.** Oxalate-supported Ca²⁺ uptake was measured by using the Ca²⁺-fluorescent dye indo-1 according to the methods of O’Brien and colleagues (25) as modified by our group (40). Fluorescence measurements were made on a spectrofluorometer (RatioMaster system, Photon Technology International) equipped with dual-emission monochromators. The measurement of \([\text{Ca}^{2+}]_0\) using the indo-1 procedure is based on the difference in the maximal emission wavelengths between the Ca²⁺-bound form of indo-1 and the Ca²⁺-free form. The excitation wavelength was 355 nm, and the emission maxima were 485 and 405 nm for Ca²⁺-free (G) and Ca²⁺-bound (F) indo-1, respectively (24). Photon counts per second were recorded simultaneously for both emission wavelengths. The Ca²⁺-independent (background) fluorescence was measured in the reaction medium (without indo-1) at each emission wavelength before the experiment was started. Background fluorescence was automatically corrected with Felix software (Photon Technology International) before the start of each assay.

The reaction buffer for the muscle homogenates contained (in mM) 200 KCl, 20 HEPES, 10 NaN₃, 0.005 N,N,N,N’-tetraakis (2-pyridylmethyl)-ethylenediamine, 5 oxalate, 15 MgCl₂, and 10 phosphenol-sulfuric acid. Before emission spectra were collected, 18 U/ml each of LDH and PK and 1.5 µM of indo 1 were added to the cuvette containing 2 ml of the reaction buffer. In addition, 3 µl of CaCl₂ (10 mM) were added at each trial to achieve an initial \([\text{Ca}^{2+}]_0\) before the start of the reaction. After 30 min, immediately after data collection was initiated, 40 µl of homogenate were added to the cuvette. This was followed by the addition of 5 mM ATP to initiate Ca²⁺ uptake. The procedures for calculating Ca²⁺ uptake are as previously described (40). Ca²⁺-uptake rates were measured over a range of \([\text{Ca}^{2+}]_0\) concentrations by using a single assay. The maximal rate of Ca²⁺ uptake for each \([\text{Ca}^{2+}]_0\) was determined by differentiating the linear fit curve. Ca²⁺ uptake rates were expressed at concentrations of 250, 500, 1,000, 1,500, and 2,000 mM. Our laboratory has found that with this procedure there is a rapid initial drop in \([\text{Ca}^{2+}]_0\). A large component of the initial drop in \([\text{Ca}^{2+}]_0\) has been attributed to Ca²⁺-binding with proteins in the homogenate (31). As a consequence, the initial changes in \([\text{Ca}^{2+}]_0\) were not used in the calculation of Ca²⁺ uptake.

It should be noted that an ATP-regenerating assay was used in the measurement of both Ca²⁺-ATPase activity and Ca²⁺ uptake. It is possible that significant loss of ADP via the adenylate kinase reaction could reduce the rate of NADH oxidation, leading to an underestimate of Ca²⁺-ATPase activity and Ca²⁺ uptake. However, this does not appear to occur because we have found no differences when an adenylate kinase inhibitor was used (unpublished observations).

Ca²⁺ release. Ca²⁺ release was measured on muscle homogenates in conjunction with Ca²⁺ uptake according to the methods of Russel et al. (31) with minor modifications (40). Ca²⁺ release was measured in a single assay, immediately after Ca²⁺ uptake. After active loading by the SR when the \([\text{Ca}^{2+}]_0\) declined to a plateau, 3 µl of AgNO₃ were added to...
give a final concentration of 141 μM. The reaction was then allowed to proceed for ~3 min. With the addition of AgNO₃, 
Ca²⁺ release generally proceeded in two phases as observed by our group (40) and previously by Ruell et al. (31). There 
was an initial rapid release (phase 1) followed by a slower, 
more prolonged rate of release (phase 2). As with the Ca²⁺ 
uptake, the curve representing [Ca²⁺]ᵢ vs. time was 
smoothed over 21 points and differentiated. The maximal 
rates of Ca²⁺ release was calculated by taking the maximum 
positive derivative for the second phase (31). Our laboratory 
has previously shown that, with AgNO₃ as the releasing 
agent, phase 2 is regulated by the Ca²⁺-release channel (39).

For Ca²⁺ uptake, Ca²⁺ release, and Ca²⁺-ATPase activity, 
protein was determined by the method of Lowry as modified 
by Schacterle and Pollock (33). In general, all Ca²⁺-ATPase 
measurements were performed separately from the Ca²⁺- 
uptake and Ca²⁺-release measurements. On a given day, the 
samples from both the control and trained legs were analyzed 
together and in triplicate.

Western blots. To assess the effects of training on Ca²⁺- 
ATPase isofrom distribution (SERCA1 and SERCA2a), electro-
phoresis and Western blotting were performed. These 
analyses were conducted on the supernatant (postnuclear 
homogenate) obtained after a one-step centrifugation process 
prepared from frozen tissue. The homogenates were prepared 
in buffer consisting of 5 mM N-2-hydroxyethylpiperazine-N'- 
2-ethanesulfonic acid (pH 7.5), 250 mM sucrose, 0.2% NaN₃, 
and 100 μM phenylmethylsulfonyl fluoride in a ratio of 15:1 
(vol/wt). Extraction was accomplished by adding an equal 
volume of buffer containing 10 mM sodium phosphate (pH 
7.4), 150 mM NaCl₂, 2% Triton X-100, 2% deoxycholate, 0.2% 
SDS, 200 μM phenylmethylsulfonyl fluoride, and 1,000 IU 
aprozin. This mixture was centrifuged at 10,000 g for 15 
min, and the supernatant was withdrawn and stored at 
−80°C until analyzed. Wu and Lytton (43) have reported that 
95–99% of the SERCA protein remains in the supernatant 
after this procedure.

Electrophoresis for separation of SR Ca²⁺-ATPase was 
performed (using 20 μg protein per lane) on the postnuclear 
homogenate with 7% SDS-polyacrylamide gels (Bio-Rad 
Mini-PROTEAN II), as described by Laemmli. After elec-
trophoresis and a 15-min equilibration in cold transfer buffer 
(25 mM Tris, 192 mM glycine, and 20% vol/vol methanol), the 
proteins were transferred to a polyvinylidene difluoride 
membrane (PVDF membrane, Bio-Rad) by placing the gels in 
transfer buffer and applying a high voltage (100 V) for 60 min 
(Trans-Blot Cell, Bio-Rad). Nonspecific binding sites were 
blocked with 10% skim milk powder in Tris-buffered saline 
(pH 7.5), applied overnight at room temperature. Immuno-
blotting was performed by using the primary monoclonal 
anbinders, A52 specific for human and 7E6 specific for hu-
man and rat (Affinity Bioreagents), for determination of 
SERCA1 and SERCA2a protein, respectively. Incubation 
with the primary antibodies was performed for 60 min at 
room temperature. After washing, a secondary antibody (an-
timouse IgG1 conjugated to horseradish peroxidase) was 
applied for 60 min at room temperature. Protein quantifica-
tion was performed by using densitometry and an enhanced 
chemiluminescence immunodetection procedure (Amersham-
ECL-RPN2106P1). After exposure to photographic film 
(Kodak Hyperfilm-ECL), the blot was developed for 120 s in 
Kodak GBX developing solution and fixed in Kodak GBX 
fixer. Protein was determined by using the Bio-Rad assay in 
which detergent is present. These procedures are essentially 
as outlined by Kandarian et al. (16). As with Kandarian et 
al., we have shown in preliminary experiments that the blot 
signal vs. protein loaded were in the linear range for our 
experimental conditions.

All samples for a given subject were run in duplicate on 
separate gels along with the standard on the same day. The 
values were initially expressed as a percentage of the stan-
dard and then as a percent of the untrained, control leg 
value. The value for the control leg was set at 100%. A sample 
of tissue from human female vastus lateralis, prepared as a 
postnuclear homogenate, divided into multiple aliquots and 
stored at −80°C, was used as a standard during each anal-
lysing session.

Statistical procedures. The data were analyzed with both 
one-way and two-way ANOVA procedures for repeated mea-
sures. One-way ANOVA procedures were used to examine 
the differences between the two legs (trained vs. untrained) 
on a single variable. When an additional variable was 
present (i.e., [Ca²⁺]ᵢ vs. leg), a two-way ANOVA was em-
ployed. When significant differences were found, Newman-
Keuls procedures as appropriate were used to locate differ-
ences between specific means. Significance was set at the 
0.05 level.

RESULTS

Table 1. Effects of training on kinetic characteristics 
of the Ca²⁺-ATPase activity

<table>
<thead>
<tr>
<th>Vₘₐₓ</th>
<th>pC₅₀</th>
<th>Hill Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>192 ± 11</td>
<td>6.01 ± 0.05</td>
</tr>
<tr>
<td>Trained</td>
<td>156 ± 18*</td>
<td>6.26 ± 0.07*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. Vₘₐₓ, maximal Ca²⁺-ATPase activity in μmol·min⁻¹·g protein⁻¹; pC₅₀, Ca concentration at half-
maximal activity; Hill coefficient, determined from Hill plots by 
using the relationship between Ca concentration and Ca²⁺-ATPase 
activity and the section of the curve that corresponded to 20–80% of 
maximal Ca²⁺-ATPase activity; control, untrained limb; trained, 
trained limb. *Significantly different (P < 0.05) from control.
Similar to the training-induced changes in Ca\textsuperscript{2+} uptake, adaptations were also noted to occur in Ca\textsuperscript{2+} release (Fig. 3). Compared with the control leg, the trained leg exhibited a value that was 26% lower (139 ± 11 vs. 103 ± 9.2 μmol·g protein\textsuperscript{-1}·min\textsuperscript{-1}; n = 7; P < 0.05).

Muscle protein content was not affected by the training protocol (14.1 ± 0.63 vs. 14.3 ± 2.3 mg/g).

The effect of training on SERCA isoform distribution was specific to the isoform type (Fig. 4). In contrast to SERCA2a, which was not altered with training, SERCA1 was depressed (P < 0.05) 13.2%. Representative Western blots for a typical volunteer are provided in Fig. 5.

**DISCUSSION**

As hypothesized, we have found that a 10-wk program of prolonged submaximal exercise results in adaptations to the SR in the vastus lateralis muscle. The adaptations consisted of reductions in Ca\textsuperscript{2+}-ATPase activity, Ca\textsuperscript{2+} uptake, Ca\textsuperscript{2+} release, and SERCA1 content. Collectively, these adaptations are consistent with a shift in the properties of the SR toward those typically observed in slow-twitch or type 1 fibers.

In this study, all of the SR functional measurements were performed on whole muscle homogenates prepared from tissue samples obtained by needle biopsy. The measurement of SR Ca\textsuperscript{2+}-ATPase activity can be problematic under such circumstances given the presence of other ATPases in the homogenate. However, the potential limitations of other contaminating ATPases have been circumvented by using a procedure developed by Simonides and van Hardeveld (37) and subsequently modified by Reull et al. (31) for application to human tissue. This procedure provides for the selective exclusion of other interfering ATPases. The specificity of the procedure has been clearly indicated by demonstrating the complete loss of Ca\textsuperscript{2+}-ATPase activity with cyclopiazonic acid, an inhibitor of the enzyme. Moreover, the assay is a regenerating assay in which ATP is continually produced by the addition of...
the glycolytic enzymes PK and LDH. The oxidation of NADH is measured fluorometrically. This procedure prevents the accumulation of ADP and P_i, which could inhibit the enzyme. Because the myokinase inhibitor AP5A had no effect on enzyme activity (unpublished observations), ADP loss via AMP formation would not appear important.

Although the plasticity of the SR has been demonstrated in animals to a variety of mechanically based chronic perturbations such as induced contractile activity (14), hindlimb unweighting (34), and overload induced by surgical removal of synergistic muscles (16), this appears to be one of the few longitudinal studies to examine SR changes in response to submaximal training in previously untrained humans. Adaptations at the level of the SR in response to regular exercise were expected given the extensive changes in Ca^{2+}-regulatory membrane processes and proteins that are elicited by chronic electrical stimulation. These changes are typically induced in muscle of rats (36), rabbits (14), and dogs (26) consisting of a predominance of fast-twitch fibers. In the present study, the nature of the adaptations that occurred in the SR in specific fiber types is unknown, given the mixed distribution of fiber types in the vastus lateralis. It should be emphasized that not all studies report adaptations in the SR in human muscle with regular submaximal exercise. In preliminary work, our laboratory failed to detect changes in maximal Ca^{2+}-ATPase activity (11), whereas others have failed to observe changes in Ca^{2+}-ATPase concentration (as assessed by Ca^{2+}-dependent steady-state phosphorylation) (21). Although the reasons underlying the contradictory findings remain unclear, particularly with regard to our laboratory’s initial study in this area, the fact that previously trained volunteers were used by Madsen et al. (21) might explain the lack of an effect with additional training. In a recent study (19), selected SR properties in the vastus lateralis muscle were compared between untrained control subjects and endurance-trained and resistance-trained athletes. Compared with untrained control subjects, endurance-trained athletes demonstrated lower Ca^{2+} uptake and Ca^{2+} release. Given the lower type II fiber-type distribution observed in the endurance-trained athletes and the fact that endurance training does not appear to induce significant shifts in the major fiber types (32), these results suggest that the differences observed in SR functional measurements might, in large part, be explained by inherent differences in major fiber-type distribution.

The most inviting mechanism to explain the adaptations that resulted in the SR in response to training is a reduction in the content of specific proteins. A reduction in Ca^{2+}-ATPase protein content is clearly indicated from the Western blot analyses and densitometric measurements in which a relative reduction in SERCA1, the isoform typically observed in fast-twitch muscle, occurs with training but not in SERCA2a, the predominant isoform in slow-twitch muscle. The reduction in Ca^{2+}-ATPase protein level was accompanied by a reduction in V_max that was observed in the trained leg compared with the control leg. It is noteworthy that the decrease in V_max occurred in the absence of changes in the Hill coefficient, a measure of the Ca^{2+}-binding sensitivity. In contrast to the Hill coefficient, a small but significant increase in pCa_{50} was noted with training. This finding indicates that, after training, a lower [Ca^{2+}]_i level can recruit 50% of V_max. Interestingly, we have also found that the training program resulted in a significantly lower homogenate [Ca^{2+}]_i in the trained leg compared with the control leg (unpublished observations).

Reductions in Ca^{2+}-ATPase protein levels could also explain the rightward shift in [Ca^{2+}]_i vs. Ca^{2+} uptake relationship after training, including the reduction in maximal Ca^{2+} uptake. Because no difference in the coupling ratio was observed between the trained and untrained legs, the apparent downregulation in Ca^{2+}-ATPase did not compromise the efficiency of Ca^{2+} transport into the SR. Our findings would also suggest that training did not result in increased vesicle Ca^{2+} leakiness, abnormal enzyme expression, or changes in relative phospholipid content of the SR membrane, because all of these factors would be expected to alter coupling ratios (22).

A coordination in SR coupling adaptations to aerobic training is also suggested in Ca^{2+} uptake and Ca^{2+} release because the reductions observed in Ca^{2+} uptake were also accompanied by reductions in Ca^{2+} release. Functionally, cooperative changes in these processes could help maintain the [Ca^{2+}]_i-time integral that occurs during activation because the reduced rate of Ca^{2+} release is also paralleled, at least qualitatively, by reductions in Ca^{2+} uptake kinetics. Reduced Ca^{2+} cycling, as suggested by the coupled changes in Ca^{2+} release and sequestration, could conceivably preserve [Ca^{2+}]_i-time integral and activation of the myofibrillar complex and force generation (pCa-force relationship) but at lower energy cost (38).

It should be emphasized that the interpretation of the effects of training on Ca^{2+} release may be influenced by the type of releasing agent employed. We have followed the procedure used by Ruell et al. (31), in which AgNO_3 is used as the releasing agent. In recent work, we have found that, unlike 4-chloro-m-cresol, which is specific to the Ca^{2+}-release channel, AgNO_3 can also act on the Ca^{2+}-ATPase and can lead to reversal of the pump action (39). Moreover, because
Ca\(^{2+}\) release occurs in two phases, training effects may be different between the phases. This study was completed before our examination of the Ca\(^{2+}\)-release kinetics using different releasing agents (39). As such, we have followed the popular practice of using the second phase of Ca\(^{2+}\) release and the releasing agent AgNO\(_3\). Additional study is warranted investigating the possibility that the effect of training may be modified depending on the releasing agent used and the Ca\(^{2+}\) release phase examined.

It should also be noted that limitations exist with regard to the assessment of Ca\(^{2+}\)-release kinetics, particularly with regard to the role of different Ca\(^{2+}\)-binding proteins. Ca\(^{2+}\)-binding proteins such as troponin-C and Ca\(^{2+}\)-ATPase would be expected to bind some of the Ca\(^{2+}\) and potentially affect the slope of [Ca\(^{2+}\)]\(_{r}\) vs. time (41). As a consequence, even though our results have been normalized to total protein content, changes in specific Ca\(^{2+}\)-binding proteins with training could potentially modify our conclusions regarding the effects of training on SR Ca\(^{2+}\)-release function per se.

A particularly important issue is the mechanisms(s) underlying the reduction is maximal Ca\(^{2+}\)-ATPase activity. Reductions in function could occur because of reductions in protein, reductions in protein function, or both. Previous studies have clearly demonstrated that prolonged induced contractile activity can result in initial reductions in maximal Ca\(^{2+}\)-ATPase activity at least in fast muscle as a result of structural alterations in the region of the adenine nucleotide binding site, whereas protein content is unchanged (5). Although the effect of voluntary exercise on Ca\(^{2+}\)-ATPase activity remains controversial, particularly in rats (7), studies have reported reductions in muscles composed of a predominance of fast-twitch fibers (4, 42), presumably as a result of inactivation of the nucleotide binding site (18). Such a mechanism also appears to exist during prolonged exercise in humans because several studies have reported disturbances in Ca\(^{2+}\)-ATPase activity in the vastus lateralis (2, 10, 15). Reductions in Ca\(^{2+}\)-ATPase activity also appear to be accompanied by reductions in Ca\(^{2+}\) uptake (2, 15). In addition, reductions in Ca\(^{2+}\) release also appear to accompany the exercise-induced reductions in Ca\(^{2+}\)-ATPase activity (6, 15, 42). The length of time that the depressions in the Ca\(^{2+}\)-ATPase activity, Ca\(^{2+}\) uptake, and Ca\(^{2+}\) release persist when contractile activity has stopped remains uncertain. In this study, a period of ~4 days was provided after the last training session before the post-training tissue sample was obtained.

The adaptations that we have observed in the properties of the SR do not appear to be linked to modifications in the major fiber-type distribution in the vastus lateralis because we have found, using both myosin-based histochemistry and myosin heavy chain analyses by Western blot techniques, that no differences existed in these properties between the trained and untrained legs (unpublished observations). Previous studies using CLFS in animals to investigate SR plasticity have utilized muscles of a predominant type II composition. The vastus lateralis in untrained humans is typically composed of an approximately equal percentage of type I and type II fibers (32), and both fiber-type populations would be expected to be activated during the training sessions (32). On the basis of the dramatic changes in SR protein expression and function that have been observed in animal type II-based muscles to CLFS, it might be expected that a large percentage of the SR adaptations observed in our study occurred in the type II fibers of the vastus lateralis and particularly those with a low oxidative potential. This hypothesis is supported by the findings of Kim et al. (17), who found that reductions in SR Ca\(^{2+}\) uptake follow treadmill running in rats were restricted to the superficial portion of the vastus lateralis, a region composed primarily of fast, low-oxidative fibers.

With our experimental design, SR alterations with training were assessed by comparing the trained leg with the untrained or control leg only at the end of the 10-wk program. This one-leg design is the model employed with CLFS. Although changes in SR expression could conceivably occur in the inactive leg as a result of hormonal influences, this does not appear to occur, at least in CLFS, in which the induced-contractile activity represents the dominant signal regulating protein expression (30). The use of time-course measurements, as has been used in CLFS, would have provided important insights not only into the pattern of changes in specific SR proteins and processes but also into the cooperative nature of the expression of multiple proteins. As an example, it has been reported that cardiac-like replacement of proteins occurs early in CLFS (9). The reductions that were observed in Ca\(^{2+}\) release in this study could be due not only to a downregulation in total RyR1 protein but to an isoform shift. Increases in RyR2, which predominates in the heart, have been found to increase in fast-twitch-based muscle with CLFS (9). Unfortunately, because of tissue limitations, we could not measure RyR protein content. With respect to Ca\(^{2+}\)-ATPase, our results indicate a reduction in protein levels with training that is due to a reduction in SERCA1 because no change was found for SERCA2a. These findings are consistent with the CLFS model, in which changes in SERCA1 precede changes in SERCA2a (27). The reduction in SERCA 2 protein occurred in the absence of changes in total muscle protein. This could occur by increased expression of other proteins. The mitochondrial protein, as an example, is known to be increased by endurance training (32). Because no changes were found in the Hill coefficient with training and only small changes in pCa\(_{50}\) were observed, the isofrom shift would appear to have minimal consequence on the Ca\(^{2+}\) sensitivity of the Ca\(^{2+}\)-ATPase. This conclusion is consistent with previous work in which it has been reported that SERCA isoforms expressed in COS cells displayed similar Ca\(^{2+}\) affinities (20).

Because both SR Ca\(^{2+}\) uptake and Ca\(^{2+}\) release may be altered by a variety of SR proteins other than SERCA and RyR, it is possible that altered expression of these proteins could have influenced the SR Ca\(^{2+}\)-
handling changes that we observed with training. A number of proteins such as the dihydropyridine recep-
tor in the t tubule and triadin in the SR couple with
RyR, and these demonstrate a similar pattern of down-
regulation with long-term CLFS (9, 14). Similarly,
changes in phospholamban in conjunction with SERCA
isoform could alter Ca2+-ATPase dependency. However,
because the effect of phospholamban is restricted only
to SERCA2a and because we found no change in
SERCA2a with training, changes in phospholamban
expression should be of little consequence on Ca2+-
sequestration properties (9).

**Perspectives.** The results of this study have several
potentially important functional implications. First
and foremost, it is apparent that, like many other
proteins of the muscle cell, regular voluntary contrac-
tile activity can also alter proteins involved in SR Ca2+
handling. Our results also suggest that when the train-
ing is prolonged and of submaximal intensity, such
that aerobic metabolism represents the dominant
source of ATP supply, the adaptations favor a reduc-
tion in the proteins and processes involved in Ca2+
handling. These changes observed in the SR are con-
sistent with reduced Ca2+-cycling kinetics similar to
what is observed in slow-twitch fibers. Reduced cycling
kinetics would be expected to allow isometric or low-
velocity activities to be performed with increased effi-
ciency. Because the coupling ratio was not altered by
the training, this could occur primarily as a product of
the decreased cycling rate per se and not by altering
the energy required per unit Ca2+ transport. In addi-
tion, because it is well documented that aerobic-based
training leads to an upregulation in mitochondrial po-
tential, the downregulation in SR Ca2+-ATPase could
lead to a more favorable balance between the utiliza-
tion of the ATP by the SR Ca2+-ATPase and aerobic
delivery of ATP. As a consequence, cellular energy
homeostasis may be better protected during prolonged
exercise.

Special appreciation is extended to Dr. Jing Ouyang for expert
technical support.

Financial assistance for the study was provided by grants to H.
Green and D. MacDougall from the National Sciences and Engineer-
ing Research Council of Canada.

**REFERENCES**

1. Berchtold MW, Brinkmeier H, and Müntener M. Calcium ion in skeletal muscle: its crucial role for muscle function, plas-


3. Briggs FN, Lee KF, Feher JJ, Wechsler AS, Ohlendieck K, and Campbell KP. Ca-ATPase isoform expression in sarcoplasm-
ic reticulum is altered by chronic stimulation of skeletal mus-


5. Dux L, Green HJ, and Pette D. Chronic low-frequency stim-
ulation of rabbit fast-twitch muscle induces partial inactivation of the sarcoplasmic reticulum Ca2+-ATPase and changes in its

6. Favero TG, Pessahin DP, and Klug GA. Prolonged exercise reduces Ca2+-release in rat skeletal muscle sarcoplasmic reticu-

7. Ferrington DA, Reineveld JC, Bär PR, and Bigelow DJ. Activation of the sarcoplasmic reticulum Ca2+-ATPase induced by

8. Franzini-Armstrong C and Protasi F. Ryosamide receptors of
striated muscles: a complex channel capable of multiple interac-

9. Froemming GR, Murray BE, Harmon S, Pette D, and
Ohlendieck K. Comparative analysis of the isoform expression
pattern of Ca2+-regulatory membrane proteins in fast-twitch,
slow-twitch, cardiac, neonatal and chronic low-frequency stimu-

10. Green HJ, Grange F, Chin C, Goreham C, and Ranney D.
Exercis-induced decreases in sarcoplasmic reticulum Ca2+-
ATPase activity attenuated by high-resistance training. *Acta

11. Green HJ, Grange F, Goreham C, Shoemaker K, and

12. Green HJ, Klug GA, Reichmann H, Seedorf U, Wieher W, and Pette D. Exercis induced fibre type transitions with re-

Ca2+-indicators with greatly improved fluorescence properties.

14. Hicks A, Ohlendieck K, Göpel SO, and Pette D. Early functional and biochemical adaptations to low-frequency to low-


16. Kandarian SC, Peters DG, Taylor JA, and Williams JJH. Skeletal muscle overload upregulate the sarcoplasmic reticulum

17. Kim DH, Wible GS, Witzmann FA, and Fitts RH. The effect of exercise training on sarcoplasmic reticulum function in fast

18. Klebi BM, Ayoub TA, and Pette D. Protein oxidation, tyrosine
nitration, and inactivation of sarcoplasmic reticulum Ca2+-

19. Li JL, Wang XN, Fraser SF, Carey MF, Wrigley TV, and
McKenna MJ. Effects of fatigue and training on sarcoplasmic reticulum Ca2+ regulation in human skeletal muscle. *J Appl

20. Lytton J, Westlin M, Burk SE, Shull GE, and MacLennan
DH. Functional comparisons between isoforms of the sarcoplas-
mic or endoplasmic reticulum family of calcium pumps. *J Biol

endurance training on the concentration of Na,K-ATPase and
Ca-ATPase in human skeletal muscle. *Acta Physiol Scand* 150:

22. Martonosi AN. Structure-function relationships in the Ca2+-
ATPase of sarcoplasmic reticulum: facts, speculations and ques-

23. Melissa L, MacDougall JD, Tarnopolsky MA, Capriano N,
and Green HJ. Skeletal muscle adaptations to training under

24. O’Brien PJ. Calcium sequestration by isolated sarcoplasmic reticulum: real time monitoring using ratiometric dual emission


