Paradoxical effects of prior activity on human sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase response to exercise

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Tupling, A. R., H. J. Green, B. D. Roy, S. Grant, and J. Ouyang. Paradoxical effects of prior activity on human sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase response to exercise. J Appl Physiol 95: 138–144, 2003; 10.1152/japplphysiol.00483.2002.—To investigate the effects of intermittent heavy exercise (HE) on sarcoplasmic reticulum (SR) maximal Ca\textsuperscript{2+}-ATPase activity ($V_{\text{max}}$) and Ca\textsuperscript{2+} uptake, a continuous two-stage standardized cycling test was performed before and after HE by untrained men [peak aerobic power ($V_{\text{O2 peak}}$) = 42.9 ± 2.7 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}]. The HE consisted of 16 bouts of cycling performed for 6 min each hour at 90% $V_{\text{O2 peak}}$. Tissue was obtained from the vastus lateralis by needle biopsy before and during each cycle test. Before HE, reductions ($P < 0.05$; μmol·g\textsuperscript{-1}·min\textsuperscript{-1}) of 16 and 31% were observed in $V_{\text{max}}$ and Ca\textsuperscript{2+} uptake, respectively, after 40 min of the standardized test. Resting $V_{\text{max}}$ and Ca\textsuperscript{2+} uptake were depressed ($P < 0.05$) by 19 and 30%, respectively, when measured 36–48 h after HE. During the standardized test, after HE, $V_{\text{max}}$ increased ($P < 0.05$) by 20%, whereas no change was observed in Ca\textsuperscript{2+} uptake. The HE protocol resulted in small increases ($P < 0.05$) and decreases ($P < 0.05$) in sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA) 2a and SERCA1 expression, respectively, as determined by Western blotting techniques. These results indicate that SR Ca\textsuperscript{2+}-sequestering function in response to a prolonged exercise test depends on prior activity status, such that rested muscles exhibit a decrease and prior exercised muscles, an increase in Ca\textsuperscript{2+}-ATPase activity. Moreover, it appears that changes in SERCA content can occur in response to a sustained session of intermittent exercise.

Ca\textsuperscript{2+} sequestering; continuous exercise; fatigue; vastus lateralis; SERCA isoforms

The sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA) is a 110-kDa integral membrane protein with known sequence and structure of 997 amino acid residues (35). Two isoforms of the Ca\textsuperscript{2+}-ATPase predominated in adult skeletal muscle, namely, SERCA1 and SERCA2a. SERCA1 accounts for >99% of the SERCA isoforms expressed in adult fast-twitch skeletal muscle, whereas SERCA2a is highly expressed in slow-twitch skeletal muscle and heart (47). The Ca\textsuperscript{2+}-ATPase translocates 2 mol Ca\textsuperscript{2+} across the sarcoplasmic reticulum (SR) membrane, against a concentration gradient, from the cytoplasm into the SR lumen, at the expense of 1 mol ATP.

In skeletal muscle, the SR Ca\textsuperscript{2+}-ATPase performs at least two crucial functions. In resting muscle, the SR Ca\textsuperscript{2+}-ATPase is responsible for maintaining cytoplasmic free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) near 100 nM. During repetitive muscle contractions, the SR Ca\textsuperscript{2+}-ATPase must rapidly sequester large Ca\textsuperscript{2+} loads from the cytoplasm into the lumen, thereby inducing muscle relaxation and effectively restoring SR Ca\textsuperscript{2+} stores. In the presence of disturbances in SERCA enzyme function, the muscle is unable to sustain repetitive muscle contractions, as a consequence of the disturbance in [Ca\textsuperscript{2+}]f and the prolongation in relaxation time (9).

There is substantial evidence to indicate that repetitive contractile activity may impair SR Ca\textsuperscript{2+} handling in skeletal muscles. In chronic low-frequency stimulation (CLFS), as an example, which is typically applied to fast-twitch (type II) muscles, for periods up to 24 h/day, pronounced reductions in Ca\textsuperscript{2+}-ATPase activity occur relatively early in the stimulation period (23, 40, 41). The reductions in Ca\textsuperscript{2+}-ATPase activity occur in the absence of changes in protein or SERCA isoform expression (40). The reductions in Ca\textsuperscript{2+}-ATPase activity appear to occur as a result of structural alterations in the region of the nucleotide binding site on the enzyme (10, 37) secondary to increases in oxygen free radicals and protein nitrosylation (29). With this model, pronounced reductions in Ca\textsuperscript{2+} uptake occur (41), accompanied by prolongation in relaxation time (41). Intracellular measurements of [Ca\textsuperscript{2+}]f demonstrate both a prolonged and depressed amplitude of the [Ca\textsuperscript{2+}]f transient (5). The depression in Ca\textsuperscript{2+} uptake persists for at least 2 days after the cessation of stimulation (31, 36).

Reductions in Ca\textsuperscript{2+}-ATPase activity and/or Ca\textsuperscript{2+} uptake have also been documented after sustained running in rats (3, 33), humans (2, 18), frogs (46), and horses (4). The effects of voluntary activity appear to depend, at least in part, on the characteristics of the task because reductions in Ca\textsuperscript{2+}-sequestering function is not always a consistent finding (6, 8, 13). In humans, there is general agreement that prolonged heavy to moderate exercise results in reductions in Ca\textsuperscript{2+}-sequestering properties in skeletal muscle (2, 15, 32, 45), presumably as a result of alterations in the region of

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the nucleotide binding site (33), similar to what is observed with CLFS (10, 37).

In previous research from our laboratory, we have shown that rapid adaptations can occur in a variety of cellular proteins and processes in human muscle if the exercise stimulus is sufficiently severe. Improvements in muscle energy homeostasis during exercise with concomitant reduction in selected by-products can be observed within the first 3 days of daily prolonged exercise in previously untrained volunteers (19). Early adaptations are also evident in the monocarboxylate transporters (MCT), MCT1 and MCT4 (20), and in the sarcoplasmic Na\(^+\)-K\(^+\)-ATPase pump (16). We have also found that intermittent heavy exercise appears to be a potent stimulus because we could induce adaptations in muscle exercise metabolism that are at least qualitatively similar to the trained state with 6 min of exercise performed at ~90% of peak aerobic power (\(\dot{V}O_2\)peak), once per hour for 16 h (21).

Our observations with the short-term training models invites the question of whether adaptations might also extend to the SR and, particularly, the inactivation that occurs in Ca\(^{2+}\)-ATPase activity with prolonged heavy exercise. Although alterations in expression at the protein level may not occur to effect a different response in Ca\(^{2+}\)-ATPase activity to prolonged exercise, alterations in the intracellular environment such as an improved phosphorylation potential and less by-product accumulation may provide protection from structural damage (4). The 6-min repetitive sessions of heavy exercise may be particularly potent in protecting enzyme function during subsequent exercise because large perturbations in Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) uptake occur (4). Moreover, this type of exercise results in a pronounced thermal stress with muscle temperatures, which, in the case of horses, reach 43°C (4). Incubation of SR vesicles (7, 14) or homogenates (43) at this temperature is known to induce large perturbations in Ca\(^{2+}\) handling with loss of coupling efficiency. It is known that cells preexposed to thermal stress quickly acquire a resistance, an effect possibly mediated by increased expression of heat shock proteins (HSPs) (26, 39). Interestingly, it has been shown that a single bout of heavy exercise is sufficient to rapidly upregulate selective HSPs (12, 28).

The purpose of the present study was to examine the role of prior activity on exercise-induced alterations in SERCA function in human muscle. Specifically, we examined whether a single, extended session of intermittent heavy exercise (HE), which is known to induce muscle metabolic adaptations (21), could protect against disturbances in Ca\(^{2+}\)-sequestering properties that occur during a prolonged exercise test. We have hypothesized that the HE sessions would result in less of a time-dependent disturbance in SR Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) uptake during exercise. Moreover, we have also postulated that these protective effects would occur in the absence of changes in SERCA protein and isoform content.

**METHODS**

**Subjects.** In total, nine healthy, male volunteers, who were active but not exercising on a regular basis (i.e., less than one per week), were recruited for the study. The physical characteristics of the subjects were as follows: age, 21 ± 0.6 (SE) yr; height, 180 ± 2.1 cm; and weight, 79.6 ± 3.6 kg. Maximal aerobic power (\(\dot{V}O_2\)peak), as determined during a progressive cycle test to fatigue, was 3.38 ± 0.17 l/min and 42.9 ± 2.7 ml·kg\(^{-1}\)·min\(^{-1}\). As required, the study was approved by the Office of Human Research and Animal Care, and all volunteers were made fully aware of all procedures before written consent was obtained.

**Experimental design.** Details of the experimental design have been reported previously (21). Briefly, nine subjects reported to the laboratory on five occasions, beginning ~2 wk before HE. On the first visit, \(\dot{V}O_2\)peak was measured. On the second visit, the subjects cycled for a brief period at the individual work rates that were to be used during a standardized two-step cycling protocol, administered before and after HE. During the third and fifth visits, the subjects performed the standardized tests. The standardized test was used to evaluate the effect of HE. On the fourth visit, HE was performed. The standardized tests were performed ~48 h before (Pre) and ~36–48 h after (Post) HE (Fig. 1).

The standardized tests consisted of cycling for 20 min at each of two work rates, namely, 60 ± 0.9 and 75 ± 1.0% of

![Fig. 1. Experimental design used to investigate the effects of heavy intermittent exercise on sarcoplasmic reticulum function. Pre test, standardized 2-stage cycling test performed before (~48 h) heavy exercise; Post test, standardized 2-stage cycling test performed after (36–48 h) the heavy exercise; %\(\dot{V}O_2\)peak, percentage of peak aerobic power; B, biopsy.](http://jap.physiology.org/content/jappl/95/4/139.supp)
VO_2_{peak}. The same absolute work rate was used on both testing occasions. Before each exercise test, the thighs of each subject were prepared for needle biopsy sampling (1). Tissues were extracted from the vastus lateralis immediately before the exercise, after the subject had been sitting quietly on the cycle for ~15 min, and at 3, 20, and 40 min of exercise. Two biopsies were performed at each site. Sampling sites were randomized between legs for each exercise test. The first biopsy sample was later analyzed for muscle metabolites (21), and a second biopsy, extracted from the same site, was used for analyses of SR function but only at rest and at 20 and 40 min of exercise. This sample was quickly extracted from the biopsy needle, immediately homogenized, and frozen in liquid N_2. On average, exercise was not interrupted for any longer than 30 s when samples were obtained. Muscle samples were stored at ~80°C until analyses.

Before and during the standardized exercise tests, respiratory gas collection was performed according to previous published methods (25) over 4- to 5-min segments beginning at 15 and 35 min of exercise. These measurements were used for determinations of O_2 uptake (VO_2). An electronically braked cycle ergometer (model 870, Quinton), calibrated to ensure full back inhibition of the Ca^2^{+} for 16 h at 90% V_\text{maxima} wavelengths for the Ca^2^{+}-ATPase was performed (using 20 μg protein per lane) on the postnuclear homogenate with 7% SDS-polyacrylamide gels (Mini-PROTEAN II, Bio-Rad) as described by Laemmli (30). After electrophoresis and equilibration (15 min) in a cold transfer buffer (25 mM Tris, 192 mM glycine, and 20% vol/vol methanol), the proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad) by placing the gel in a transfer buffer and applying a high voltage (100 V) for 60 min (Trans-Blot Cell, Bio-Rad). Nonspecific binding sites were blocked by 10% skim milk powder in Tris-buffered saline (pH 7.5). Identification of SERCA1 and SERCA2a was accomplished by immunoblotting using the primary monoclonal antibodies MA3–912 and MA3–919 (Affinity Bioreagents, Golden, CO). Protein quantification, after application of a secondary antibody (anti-mouse IgG1 conjugated to horseradish peroxidase), was performed by densitometry and an enhanced chemiluminescence immunodetection procedure (ECL-RPN2106P1, Amersham, Baie d’Urfe, PQ, Canada). Protein was determined by using the Bio-Rad assay with detergent. In preliminary work, we established the linearity between the blot signal and protein loaded. All samples for a given individual were run in duplicate on separate gels along with the standard on the same day. A sample of tissue prepared as a postnuclear homogenate from the vastus lateralis of an untrained volunteer and divided into multiple aliquots was stored at ~80°C and served as the standard. The values were initially expressed as a percentage of the standard and then as a percentage of the initial Pre-HE value. The Pre-HE value was set at 100%. All procedures were identical to those recently published by our laboratory (11).

**Analytic procedures.** Measurements of maximal Ca^{2+}-ATPase activity and submaximal Ca^{2+} uptake were performed on muscle homogenates with the use of a 11:1 (vol/wt) dilution of buffer containing (in mM) 200 sucrose, 40 t-histidine, 1 EDTA, 10 NaNO_3, and 1 dithiothreitol (pH 7.8), using a hand-held glass-glass homogenizer (Daul 20, Kontes) (38). The specific assays employed were according to Simonides and van Hardeveld (44) and O’Brien (38) for Ca^{2+}-ATPase activity and Ca^{2+} uptake, respectively, with minor modifications as previously described by our group (45).

Total Ca^{2+}-ATPase activity was measured spectrophotometrically (UV 160U, Shimadzu) over a range of [Ca^{2+}]_r between 6.6 and 16.1 μM, the range that elicits maximal Ca^{2+}-ATPase activity (44). Basal or Mg^{2+}-ATPase activity was measured at a [Ca^{2+}]_r of 17 mM, the level necessary to ensure full back inhibition of the Ca^{2+}-ATPase enzyme activity (44). The SR Ca^{2+}-stimulated ATPase activity is based on the difference between the total and basal ATPase activities.

Oxalate-supported Ca^{2+}-uptake rates were measured by using the Ca^{2+} fluorescent dye indo-1. Fluorescence measurements were made on a spectrofluorometer (Ratio Master Spectrofluorometer, Photon Technology International) equipped with dual-emission monochromators. The dual-emission fluorometer allows simultaneous measurements of Ca^{2+}-bound and Ca^{2+}-free forms on the basis of the difference in emission maxima wavelengths for the Ca^{2+}-free and Ca^{2+}-bound to indo-1 (38). The rate of Ca^{2+} uptake was determined at a [Ca^{2+}]_r of 750 nM. Unfortunately, this level was considerably below the level needed to measure maximal Ca^{2+} uptake and the level employed to measure maximal Ca^{2+}-ATPase activity.

For both the Ca^{2+}-ATPase activity and Ca^{2+} uptake, protein was determined by the method of Lowry as modified by Schacterle and Pollock (42). On a given day, all samples for a given variable and for a given individual were analyzed in duplicate.

**Electrophoresis and Western blotting.** Electrophoresis and Western blotting were performed on postnuclear homogenates after centrifugation and extraction of the supernatant (n = 7). Homogenates from frozen tissue were prepared as previously described (27). Electrophoresis for isolation of the SR Ca^{2+}-ATPase was performed (using 20 μg protein per lane) on the postnuclear homogenate with 7% SDS-polyacrylamide gels (Mini-PROTEAN II, Bio-Rad) as described by Laemmli (30). After electrophoresis and equilibration (15 min) in a cold transfer buffer (25 mM Tris, 192 mM glycine, and 20% vol/vol methanol), the proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad) by placing the gel in a transfer buffer and applying a high voltage (100 V) for 60 min (Trans-Blot Cell, Bio-Rad). Nonspecific binding sites were blocked by 10% skim milk powder in Tris-buffered saline (pH 7.5). Identification of SERCA1 and SERCA2a was accomplished by immunoblotting using the primary monoclonal antibodies MA3–912 and MA3–919 (Affinity Bioreagents, Golden, CO). Protein quantification, after application of a secondary antibody (anti-mouse IgG1 conjugated to horseradish peroxidase), was performed by densitometry and an enhanced chemiluminescence immunodetection procedure (ECL-RPN2106P1, Amersham, Baie d’Urfe, PQ, Canada). Protein was determined by using the Bio-Rad assay with detergent. In preliminary work, we established the linearity between the blot signal and protein loaded. All samples for a given individual were run in duplicate on separate gels along with the standard on the same day. A sample of tissue prepared as a postnuclear homogenate from the vastus lateralis of an untrained volunteer and divided into multiple aliquots was stored at ~80°C and served as the standard. The values were initially expressed as a percentage of the standard and then as a percentage of the initial Pre-HE value. The Pre-HE value was set at 100%. All procedures were identical to those recently published by our laboratory (11).

**RESULTS**

**Respiratory gas exchange.** As expected, progressive increases in VO_2 were observed in response to the two intensities of exercise that were employed during the standardized cycle protocol. Exercise VO_2 was anal-
with rest, the increase in maximal Ca\textsuperscript{2+}-ATPase activity observed in the initial 20 min was not significant. Before and after HE, basal or Mg\textsuperscript{2+}-ATPase remained unaltered (data not shown).

**Ca\textsuperscript{2+} uptake.** Exercise also resulted in a progressive depression (P < 0.05) in Ca\textsuperscript{2+} uptake, which was observed at a [Ca\textsuperscript{2+}]\textsubscript{i} of 750 nM (Fig. 2B). The decreases in Ca\textsuperscript{2+} uptake amounted to 18 and 31% of preexercise values at 20 and 40 min, respectively. As with maximal Ca\textsuperscript{2+}-ATPase activity, resting measures of Ca\textsuperscript{2+} uptake were 30% lower (P < 0.05) after HE compared with before HE. However, after HE, Ca\textsuperscript{2+} uptake was not altered with exercise (Fig. 2B).

**Western blotting.** Western blotting was performed on postnuclear homogenates prepared from frozen tissue sampled at rest, both 48 h before and ~36–48 h after HE. The SERCA2a isoform, expressed in slow-twitch muscle, was slightly increased (P < 0.05) after HE, whereas the SERCA1 isoform, expressed in fast-twitch muscle, was depressed (P < 0.05) by ~9% (Fig. 3).

**DISCUSSION**

In this study, we examined whether the response of the Ca\textsuperscript{2+}-ATPase and Ca\textsuperscript{2+} uptake to continuous cycle exercise would be similar before and after a single heavy exercise session, involving 6 min of exercise each

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**Fig. 2.** Sarcoplasmic reticulum maximal Ca\textsuperscript{2+}-ATPase activity (A) and Ca\textsuperscript{2+} uptake (B) during standardized exercise. These measurements were performed in homogenates that were prepared from muscle biopy samples obtained from the vastus lateralis during a 2-stage prolonged cycle test before (Pre) and 36–48 h after (Post) 16 h of intermittent heavy activity. Values are means ± SE; n = 9.

*Significantly different from 0 min, P < 0.05. †Significantly different from 0 and 20 min, P < 0.05.

**Fig. 3.** Western immunoblotting analyses of relative sarco(endo)plasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA) 1 and SERCA2a protein content in postnuclear homogenates. Postnuclear homogenates were prepared from muscle biopy samples taken from human vastus lateralis at rest (standard (Std)), before (Pre), and after (Post) the intermittent heavy exercise protocol. A: postnuclear homogenates (20 μg) from 7 subjects were separated by SDS-PAGE on 7% acrylamide gels, transferred to nitrocellulose, and stained with antibody MA3-912 against SERCA1. Samples were run in duplicate, but only 1 blot is shown. B: postnuclear homogenates (20 μg) from 7 subjects were separated by SDS-PAGE on 7% acrylamide gels, transferred to nitrocellulose, and stained with antibody MA3-912 against SERCA2a. Samples were run in duplicate, but only 1 blot is shown. C: when corrected relative to the standard and expressed relative to Pre test values, there was a 9% decrease (P < 0.05) in SERCA1a and a 7% increase (P < 0.05) in SERCA2a levels after the intermittent heavy exercise protocol. Values are means ± SE.
hour at ~90% \( V_{\text{O}_2}\text{peak} \) for 16 h. As hypothesized, both maximal Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) uptake were reduced with exercise before HE. However, unexpectedly, maximal Ca\(^{2+}\)-ATPase activity, measured at rest ~36–48 h after HE, was lower compared with normal and increased during the standardized exercise test to a level not different from normal resting values. Similarly, a reduction in Ca\(^{2+}\) uptake, measured in resting tissue before and after HE, was significant, whereas the exercise-induced increase observed post-HE was not statistically significant.

The acute exercise-induced reduction in SR Ca\(^{2+}\)-ATPase activity that we observed before HE supports previous work on humans (2, 18, 32, 45), where SR function was assessed in vitro. Other human studies (2, 15, 18, 24, 32, 45) have also shown reductions in Ca\(^{2+}\) uptake with exercise similar to this study. In studies where both Ca\(^{2+}\) uptake and Ca\(^{2+}\)-ATPase activity have been measured, the reductions in these properties with exercise appear to occur in parallel (2, 33). On the basis of the studies that used CLFS (40) and voluntary activity in rats (3), it would appear that the reductions in Ca\(^{2+}\)-ATPase activity are mediated by alterations to the nucleotide binding region of the enzyme (33). The reduction in resting Ca\(^{2+}\)-ATPase activity that occurred 36–48 h after the session of HE suggests that structural modifications were induced during the repetitive exercise bouts and persisted during this time frame.

During the standardized test after HE, maximal Ca\(^{2+}\)-ATPase activity increased to approximate the value measured at rest before HE. To our knowledge, the paradoxical behavior that we have observed in Ca\(^{2+}\)-ATPase activity has not been observed previously.

With our experimental design, it is not possible to attribute the changes in SR behavior observed during the posttesting sessions to HE because the effects of the exercise test performed before HE could have persisted. The two testing sessions were separated by 4–5 days. However, in recent work, we have used prolonged exercise to induce a reduction in Ca\(^{2+}\)-sequestering properties and repeated the test 4 days later (unpublished observations). We found no difference between the two conditions in Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) uptake either before or after exercise. These observations suggest that HE was responsible for the peculiar effects that we observed during exercise after HE.

A number of possible mechanisms exist to explain the increase in Ca\(^{2+}\) pump activity that we have observed during exercise after HE. Increased maximal Ca\(^{2+}\)-ATPase activity than observed before exercise has been observed by Ferrington et al. (13) in SR vesicle preparations during light exercise after prolonged treadmill running to fatigue in rats. The 30% increase in activity, compared with normal resting activity, was associated with an increase in the concentration of phosphorylated enzyme intermediate formed from ATP, which was interpreted to reflect an increase in the number of active pump subunits (13). Increased pump recruitment could occur as a result of changes in protein conformation and folding, allowing accessibility of the nucleotide domain and activation. An additional mechanism that could induce increases in Ca\(^{2+}\)-ATPase activity is phosphorylation of the SR Ca\(^{2+}\)-ATPase (only SERCA2a) by Ca\(^{2+}\)/calmodulin-dependent protein kinase at Ser\(^{38}\), which results in a greater than twofold increase in maximal activity (22). Because normal human muscle is composed of ~50, 35, and 15% of the type I, IIA, and IIB fibers, respectively (17), and thus would express equal amounts of SERCA1 and SERCA2a, all mechanisms remain possible in human skeletal muscle and could be involved in the exercise-induced increase in Ca\(^{2+}\)-ATPase activity that we observed after HE. However, it should be emphasized that unlike the Ferrington et al. study, in which an overshoot in maximal Ca\(^{2+}\)-ATPase activity was observed during active recovery after an exercise protocol that did not elicit reductions in Ca\(^{2+}\)-ATPase activity, our increase in maximal Ca\(^{2+}\)-ATPase activity with exercise after HE served only to reverse the depression observed at rest.

Changes in HSP expression might also be implicated in the increase in SR Ca\(^{2+}\)-ATPase activity that we have observed. It has been shown that heat shock in rats results in a 25% increase in maximal Ca\(^{2+}\)-ATPase activity in cardiac SR and protects the myocardium from ischemia-reperfusion injury (39). The authors associated the increase in SR pump activity with a 10-fold elevation in HSP72. It is well known that HSP72 and various other stress proteins are induced with acute exercise in mammalian muscle, including human muscle (12, 28). Increases in HSP72 with the HE protocol that we have used is particularly inviting because the 6-min bout of exercise would be expected to increase muscle temperature to over 40°C (4). Because 16 repetitions of this activity were performed, the muscle would be repeatedly subjected to a thermal insult. It is known that the organism can quickly increase thermotolerance when exposed to thermal stress (26).

We have also investigated whether changes in SERCA isoform type were induced by our HE protocol, which might explain some of our observations on Ca\(^{2+}\)-ATPase activity. We were surprised to find small but significant decreases in SERCA1 and increases in SERCA2a. SERCA1 and SERCA2a are predominately expressed in fast-twitch and slow-twitch skeletal muscle, respectively (47). Previous reports using CLFS in rabbit fast-muscle have concluded that alterations in protein content and isoform shifts do not occur for at least 10 days after the onset of CLFS (23, 40). The fact that we have demonstrated that significant changes in content can occur with a single session of HE suggests that the nature of the exercise stimulus and/or species is important in the onset and magnitude of the change that can be expected. The small changes that we have observed in SERCA isoform level would be expected to have little consequence on the behavior of Ca\(^{2+}\)-ATPase activity. Moreover, it has been previously shown that the primary determinant of Ca\(^{2+}\)-ATPase activity is not the isoform type but the abundance of the protein (34).
It is important to realize that the exercise-induced increase in Ca\(^{2+}\)-ATPase activity that we observed after HE was increased from a relatively low value compared with normal and was not elevated above normal resting values by the end of the two-step cycle protocol. The failure of Ca\(^{2+}\)-ATPase activity to recover to normal resting levels as a result of HE is similar to what has been observed after CLFS to rat muscle (36). The loss of catalytic activity has been attributed to irreversible inactivation of the Ca\(^{2+}\)-ATPase by protein gradation (36). As a result, it is possible that the recovery of Ca\(^{2+}\)-ATPase activity during exercise is due to an increase in recruitment of the portion of enzyme pool that was unaffected by HE.

Of particular significance in this study was the relationship between Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) uptake. Unfortunately, as a result of the limited sensitivity of the indo-1 dye at high [Ca\(^{2+}\)]\(_{i}\) levels, we could not investigate coupling ratios. Our conclusions regarding the behavior of Ca\(^{2+}\) uptake is limited to submaximal Ca\(^{2+}\) levels (i.e., 750 nM). Despite this limitation, there appears to be a reasonable parallel between the reduction in maximal Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) uptake during the initial standardized cycling task. This would imply that the reductions in Ca\(^{2+}\)-ATPase activity are mediated by the inhibition that occurs to Ca\(^{2+}\)-ATPase activity. This is not a surprising conclusion because it has been observed previously that coupling ratios are generally maintained after prolonged exercise (3, 32). Moreover, it has been reported that SR membrane integrity is unchanged with prolonged exercise (13).

During the standardized test after HE, the results are not as clear. Although depressions in both maximal Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) uptake were observed at rest, a dissociation between the two properties is suggested during exercise. During exercise, maximal Ca\(^{2+}\)-ATPase activity was observed to increase, whereas Ca\(^{2+}\) uptake remained stable. It is assumed that a dissociation did occur during exercise after HE, it could occur as a result of increased leakage, either from increased membrane permeability or the Ca\(^{2+}\)-release channel, which would reduce the net Ca\(^{2+}\) transport into the SR at unchanged Ca\(^{2+}\)-ATPase activity. It is of potential interest that a pronounced effect of thermal stress is to increase the permeability of the membrane for [Ca\(^{2+}\)]\(_{i}\) reportedly as a result of enzyme oligomerization and the formation of channels (7, 14).

In summary, both maximal Ca\(^{2+}\)-ATPase activity and submaximal Ca\(^{2+}\)-uptake were reduced with an acute cycling test. In contrast, after an extended session of intermittent exercise, the depression in maximal Ca\(^{2+}\)-ATPase activity observed at rest was reversed during performance of the cycling task. Although Ca\(^{2+}\) uptake was depressed at rest after the intermittent exercise, recovery did not occur during the standardized test. These results are novel compared with other similar exercise studies in humans and suggest that different mechanisms may be involved in causing the reduction in SR Ca\(^{2+}\) pump function with various exercise protocols. Our findings illustrate the necessity for characterizing the time course and response of the Ca\(^{2+}\)-ATPase with recovery and the mechanisms involved to understand the effects of subsequent exercise on SR Ca\(^{2+}\) pump function. Given the limitations in securing sufficient tissue by biopsy from human muscle, animal models appear essential. With an animal model, the provision of sufficient tissue will enable isolation of enriched SR fractions for in depth membrane and enzyme analysis. Moreover, by examining different muscles with different fiber type composition, insight into fiber type susceptibility and SR function with exercise can be obtained.

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REFERENCES

16. Green H, Burnett M, Roy B, Fowles J, and Grant SM. Increases in muscle Na\(^+\)-K\(^+\)-ATPase pumps precede changes in...


