Effects of progressive exercise and hypoxia on human muscle sarcoplasmic reticulum function

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Submitted 5 September 2003; accepted in final form 9 January 2004


Effects of exercise on the sarcoplasmic reticulum (SR) are known to play a central role in fatigue. However, the contribution of the SR to fatigue during exercise and the effects of hypoxia on the SR remain unclear. The purpose of this study was to determine the effect of progressive exercise in normoxia (N) and hypoxia (H) on the SR and whether alterations in SR Ca2+ cycling are related to fatigue. Exercise in N resulted in a 19% (P < 0.05) PO peak and VO2 peak, respectively. During progressive exercise in N, Ca2+-ATPase kinetics, as determined by maximal activity, the Hill coefficient, and the Ca2+ concentration at one-half maximal activity were not altered. However, reductions with exercise in N were noted in Ca2+ release (before exercise = 357 ± 29 μmol·min−1·g protein−1; at fatigue = 306 ± 26 μmol·min−1·g protein−1; P < 0.05) when measured at free Ca2+ concentration of 2 μM and in phase 2 Ca2+ release (before exercise = 716 ± 33 μmol·min−1·g protein−1; at fatigue = 500 ± 53 μmol·min−1·g protein−1; P < 0.05) when measured in vitro in whole muscle homogenates. No differences were noted between N and H conditions at comparable power output or at fatigue. It is concluded that, although structural changes in SR Ca2+-cycling proteins may explain fatigue during progressive exercise in N, they cannot explain the lower PO peak and VO2 peak observed during H.

AMONG THE EXCITATION-CONTRACTION (E-C) processes involved in translating the neural signal into force in the muscle cell, the sarcoplasmic reticulum (SR) remains as an inviting site of regulation of cytosolic free Ca2+ concentration ([Ca2+]i) and, consequently, activation of the myofibrillar complex and force generation (4).

The SR, an intracellular membranous network that envelopes the myofibrils, regulates [Ca2+]i levels by selective control of the rate of Ca2+ release from Ca2+-stored inside the SR and by the rate of Ca2+ uptake (4). Ca2+ release depends on the open state of the Ca2+-release channels (CRC) or ryanodine receptors, an ~450-kDa protein embedded in the SR membrane, most of which is localized to the terminal cisternae, which is in close apposition to the T tubules (12). It is now generally acknowledged that, in skeletal muscle, the action potentials that are conducted along the sarcolemma and into the T tubule result in a physical-chemical coupling between the T tubules and the CRC and opening of the CRC, enabling the rapid release of Ca2+ (4). The sequestration of [Ca2+]i back into the SR is controlled by the Ca2+-ATPase, an ~110-kDa protein located primarily in the longitudinal SR, which uses the energy from the hydrolysis of ATP to pump Ca2+ against its concentration gradient for storage in the SR (27). Increases in [Ca2+]i are a potent stimulus for increasing the activity of Ca2+-ATPase and, consequently, the rate of Ca2+ uptake into the SR (32).

The possibility that disturbances in SR Ca2+ cycling may be involved in fatigue during submaximal exercise is also supported by several studies that have demonstrated reductions in both Ca2+-ATPase activity (6, 7, 14), Ca2+ uptake (6, 7), and Ca2+ release (10) in both rat and human locomotor muscles. Information is emerging to indicate that the disturbances in Ca2+ uptake and Ca2+ release are mediated by structural adaptations in the Ca2+-ATPase enzyme (26) and the CRC (9) secondary to oxidation and/or nitrosylation mediated by reactive O2 species (ROS) (23, 24).

During intense dynamic exercise, increases in the rate of Ca2+ cycling in the muscle cell and consequently in the rate of Ca2+ release and Ca2+ uptake are necessary to realize increases in PO. A disturbance in either Ca2+ release and/or Ca2+ uptake would be expected to alter the [Ca2+]i integral and disrupt force profiles (1).

Intuitively, one might expect that supramaximal fatiguing exercise would produce even more dramatic reductions in SR Ca2+ cycling. There is evidence to support this idea since pronounced reductions in Ca2+-ATPase activity and Ca2+ uptake have been reported with exhaustive running in horses (8) and rats (52) and with intense knee extensions in humans (13, 25). Pronounced depressions in SR Ca2+ release have also been reported (25, 43). However, not all studies are consistent, because reports have been published indicating no change in Ca2+-ATPase activity (19, 31) and in Ca2+ uptake (18, 31) with intense, repetitive contractions.

Progressive dynamic exercise to fatigue is commonly employed to assess peak aerobic power [peak O2 consumption (VO2 peak)], one of the most important properties in applied physiology. However, it is unclear whether the peak mechanical power output (PO peak) attained is limited by a failure in SR Ca2+ cycling.

Hypoxia is known to result in a reduction in PO peak and VO2 peak during progressive exercise to fatigue (15, 33). It is...
For all tests, fatigue was defined as an inability to maintain pedal maximal or near maximal physiological response could be achieved. Incremental exercise test to fatigue during normoxia. This test was within a minimum of 3 wk between test sessions for each design was employed. In this design, all participants completed each written consent was obtained. Protocols and risks were carefully explained to each volunteer before approved by the Office of Research Ethics, and all experimental protocols and risks were carefully explained to each volunteer before written consent was obtained.

Experimental design. To investigate the effects of progressive cycle exercise in normoxia and hypoxia on muscle SR function. We have hypothesized that progressive reductions in Ca\(^{2+}\)-ATPase activity, Ca\(^{2+}\) uptake, and Ca\(^{2+}\) release would occur with increases in PO. We have also postulated that, at similar PO, the depression in the Ca\(^{2+}\)-cycling properties would be more pronounced in hypoxia compared with normoxia. However, at fatigue in normoxia vs. hypoxia, the disturbances would be comparable, consistent with the lower POpeak and V\(\dot{O}_2\)peak typically observed with hypoxia.

METHODS

Participants. Participants were nine healthy male university students who were not engaged in vigorous physical activity on a regular basis. Age, height, and body mass were 20.0 ± 0.37 yr, 175 ± 2.3 cm, and 80.0 ± 3.5 kg (means ± SE), respectively. All procedures were approved by the Office of Research Ethics, and all experimental protocols and risks were carefully explained to each volunteer before written consent was obtained.

During the actual test days, the participants reported to the laboratory ~60 min before the beginning of the exercise test. During this time, the individual was prepared for muscle biopsies. For tissue sampling, ~0.5 ml of a local anaesthetic (2% xylocaine) was administered to selected sites over the vastus lateralis muscles and then prepared for needle biopsies (5). For the test in normoxia, four separate sites were prepared balanced between the two legs. For the test in hypoxia, three separate sites were prepared, two in one leg and one in the other. After the preliminary preparations, the volunteers reported to the exercise testing area, mounted an electrically braked cycle ergometer (Siemens Elma 380 B), and were prepared for heart rate and respiratory gas-exchange measurements. These measurements were obtained continuously before and throughout exercise.

For the test conducted in normoxia, the participant breathed room air for 15 min before the beginning of exercise (Fig. 1). The exercise protocol consisted of 4 min of cycling at 25 W followed by step increases in work rate of 15 W/min until fatigue (Fig. 1). Before the start of cycling, resting tissue samples were obtained. In addition, tissue samples were obtained at mechanical POs equivalent to ~50 and 70% V\(\dot{O}_2\)peak as determined in normoxia and at fatigue. To obtain the exercise biopsies, the cycling was briefly interrupted and the tissue quickly extracted from previously prepared sites. The order of biopsies was alternated between legs.

For the test conducted in hypoxia, only three biopsies were performed, namely during rest and at the same absolute PO as in normoxia (which corresponded to 50% V\(\dot{O}_2\)peak as determined in normoxia) and at fatigue. The final exercise biopsy at fatigue in hypoxia was obtained at a PO equivalent to ~80% V\(\dot{O}_2\)peak in normoxia. As with the normoxia test, the participant sat quietly on the cycle and breathed room air for 15 min before the resting tissue sample was obtained. However, unlike the normoxia condition, the volunteer breathed a hypoxic gas mixture after tissue sampling from a Tissot tank, which was used as a gas reservoir. The gas mixture, containing 14% O\(_2\) and the balance N\(_2\), was continuously provided by premixed gas cylinders. After the tissue sampling, the hypoxic gas mixture was breathed continuously during the exercise. The rate of flow of gas into the Tissot was continuously adjusted to maintain an adequate supply of gas. Identical procedures were employed for normoxia, except that room air was used.

On a given experimental day, the participant was instructed to consume a light snack consisting of juices. Approximately 3 h before reporting to the laboratory, a meal-replacement beverage was provided (Ensure: 250 kcal; 61, 24, and 15% Kcals from carbohydrates, lipids, and proteins, respectively). This procedure was utilized to standardize the nutritional intake before the two test conditions. Before testing, water intake was not controlled. All volunteers were instructed not to consume alcohol and caffeine nor to engage in heavy exercise for at least 24 h before testing. For each subject, the two exercise tests were performed at approximately the same time of day. For respiratory gas collection, an open-circuit system was employed as previously described (21). The electronic cycle was calibrated on a daily basis, and care was taken to ensure that the seat height was
standardized for each volunteer between tests. Average environmental conditions in the testing room were not different between conditions and ranged between 50 and 60% relative humidity and 21 and 22°C in temperature.

Measurement of SR function. Immediately after extraction of the tissue, homogenates were prepared and stored at −80°C until analyses of Ca²⁺-ATPase activity, Ca²⁺ uptake, and Ca²⁺ release. Homogenates, prepared under ice-cold conditions, consisted of 1:1 (vol/vol) dilution of buffer containing (in mM) 250 sucrose, 5 HEPES, 10 NaN₃, and 0.2 PMSF (pH 7.5). Tissue (30–40 mg) was homogenized by using a handheld glass homogenizer (Kontes, Duall 20) (29). Once prepared, the homogenate was separated into a number of aliquots and quick frozen in liquid N₂ before storage. On a given analytical day and just before analysis, aliquots were thawed and processed for specific measurements. For a given property, duplicate measurements were performed. Care was taken to ensure that a complete set of measurements were made on all tissues for a given participant during a specific analytical session.

Ca²⁺-ATPase activity. Ca²⁺-ATPase activity was assessed spectrophotometrically using the basic procedures developed by Simonides and van Hardeveld (40), as modified in our laboratory (45). The reaction buffer contained (in mM) 200 KC1, 20 HEPES, 15 MgCl₂, 1 EGTA, 10 NaN₃, 5 ATP, and 10 phosphoenolpyruvate. The pH of the buffer was adjusted to 7.0 at 37°C. Just before the reaction was started, 18 U/ml lactate dehydrogenase, 18 U/ml pyruvate kinase, 0.3 mM NADH, and 25 μM of homogenate were added to a cuvette containing 1 ml of reaction buffer. One micromolar Ca²⁺ ionophore A-23187 (Sigma C-7522) was also added to prevent intraluminal Ca²⁺ accumulation in SR and inhibition of Ca²⁺-ATPase activity (40). Assays were performed in duplicate at 37°C and 340 nm (Shimadzu UV 160) using ~1 mg wet wt of tissue per assay. After the baseline absorbance of NADH was recorded for ~1 min, the reaction was initiated by adding 1 μl of 100 mM CaCl₂ and monitored for ~2 min.

Maximal Ca²⁺-ATPase activity and Ca²⁺ dependency of Ca²⁺-ATPase activity were measured using a 0.5-μl addition of 100 mM CaCl₂. The additions were continued until a plateau and subsequent decline in Ca²⁺-ATPase activity were observed. Basal or Mg²⁺-ATPase activity was determined in the presence of 40 μM of the specific inhibitor of the Ca²⁺-ATPase cyclopiazonic acid (38). Ca²⁺-ATPase activity is based on the difference between the total and basal ATPase activities. The validity of SR Ca²⁺-ATPase activity assay has been previously established in whole muscle homogenates (34, 40). It should be emphasized that a potential limitation of the regenerating assay employed is the possible loss of ADP to AMP via the myokinase reaction. However, we have shown using a myokinase inhibitor (AP5A) that Ca²⁺-ATPase activity is unaffected (unpublished observations).

Measurement of [Ca²⁺]ᵣ, which is used to assess Ca²⁺ dependency of the Ca²⁺-ATPase reaction, was assessed with dual-wave spectrophotometry and the Ca²⁺ fluorescent dye indo 1 according to procedures previously detailed (46, 48). In summary, the measurement of [Ca²⁺]ᵣ is based on the difference in maximal emission wavelengths between the Ca²⁺-bound-indo 1 complex and the Ca²⁺-free indo 1 complex. An excitation wavelength of 355 nm and the emission maxima were recorded at 405 and 485 nm for Ca²⁺-bound and Ca²⁺-free indo 1. Felix software (Photon Technology International) was used to calculate the ionized Ca²⁺ concentration according of Eq 2.5 of Grynkiewicz et al. (17). The dissociation constant that was used for the interaction between Ca²⁺ and indo 1 was 250 nM (17). It should be emphasized that in homogenates the actual dissociation constant can be significantly affected by the protein composition. As such, given the uncertainty as to the true value of the dissociation constant, the Ca²⁺ values used throughout the text are only apparent.

The kinetic properties of the Ca²⁺-ATPase activity that were measured included the maximal activity (Vₘₐₓ), the [Ca²⁺]ᵣ, needed to obtain half-Vₘₐₓ (Cₘₐₓ), and the Hill coefficient (nₗ). To obtain these properties, Ca²⁺-ATPase activity was plotted against the negative logarithm of [Ca²⁺]ᵣ (pCa). Vₘₐₓ represented the peak value, Cₘₐₓ represented the [Ca²⁺]ᵣ obtained from a sigmoid fit of the data that yields 50% of Vₘₐₓ, and nₗ was obtained through nonlinear regression with computer software (GraphPad Software) by using a portion of the curve that corresponded to between 20 and 80% of Vₘₐₓ. These procedures have been described in recent papers from our laboratory (37, 49).

Ca²⁺ uptake and Ca²⁺ release. Both Ca²⁺ uptake and Ca²⁺ release were measured during the same assay. In general, the procedure employed involved using the Ca²⁺ fluorescent dye indo 1 according to the methods of O’Brien et al. (30) as modified by our group (46). The reaction buffer for these assays contained (in mM) 200 KC1, 20 HEPES, 15 MgCl₂, 10 NaN₃, 0.005 N.N.N.N’.N’-tetraakis(2-pyridylmethyl)-ethylenediamine, 5 oxalate, and 10 phosphoenolpyruvate. Before each assay, 1.5 μM indo 1, 18 U/ml lactate dehydrogenase, and 18 U/ml pyruvate kinase were added to 2 ml of reaction buffer. In addition, 2.5 μl of CaCl₂ (10 mM) were added to the cuvette to produce a consistent starting [Ca²⁺]ᵣ of ~3.5 μM. After a constant [Ca²⁺]ᵣ was achieved, 5 mM ATP was added to the cuvette to initiate Ca²⁺ uptake. Ca²⁺ uptake rates were assessed over a range of [Ca²⁺]ᵣ by using a single assay. [Ca²⁺]ᵣ was measured as described earlier. The release phase 1 (RC₁) was determined by differentiating the linear fit curve using four concentrations of cytosolic free Ca²⁺, namely 500, 1,000, 1,500, and 2,000 μM. We have found that with this procedure there is a rapid initial drop in [Ca²⁺]ᵣ. A large component of the initial drop in [Ca²⁺]ᵣ has been credited to the binding of Ca²⁺ with proteins in the homogenates (34). As a consequence, the initial changes in [Ca²⁺]ᵣ were not used in the calculation of Ca²⁺ uptake (34).

Ca²⁺ release was measured immediately after Ca²⁺ uptake according to the general procedures of Ruell et al. (34). However, unlike Ruell et al., who employed AgNO₃, we have employed 4-chloro-m-cresol (4-CMC). We have recently shown that effects of 4-CMC are specific to the CRC and do not cause Ca²⁺-ATPase pump reversal, as was noted for AgNO₃ (47). To assess Ca²⁺-release rates, 20 μM of 4-CMC was added to the cuvette after [Ca²⁺]ᵣ declined to a plateau after the measurement of Ca²⁺ uptake. We have found that a biphasic Ca²⁺ release can be distinguished, an early release, which we have labeled phase 1, and a slower, more delayed release, which we have labeled phase 2 (47). Maximal release rates for each phase were calculated using the same method as for Ca²⁺ uptake and differentiating using a linear-fit curve.

It should be noted that, because Ca²⁺ uptake and Ca²⁺ release were measured in the same assay, the reaction buffers were the same. It is now possible that optimal assay conditions may vary with the property measured. As an example, 15 mM MgCl₂ was used for both Ca²⁺ uptake and Ca²⁺ release. In the case of Ca²⁺ release, optimal MgCl₂ levels appear to be between 20 and 30 mM (unpublished observations).

For all SR properties, protein was determined by the method of Lowry as modified by Schacterle and Pollock (36). This involved separate protein assessments in homogenate used for Ca²⁺-ATPase activity and in homogenates used for Ca²⁺-uptake and Ca²⁺-release assays. There were no significant differences in protein levels between normoxic and hypoxic conditions. The coefficients of variation for duplicate measurements for Ca²⁺ uptake and Ca²⁺-ATPase activity were 7.6 ± 0.9 and 7.9 ± 2.2%, respectively. Only single measurements were used for Ca²⁺-release determinations.

Statistics. Group data are expressed throughout the text as means ± SE. To examine the effects of progressive exercise and O₂ condition on SR function, linear regression techniques were employed, and the slopes of the regression lines for each condition were established. Student’s t-tests were used to examine differences between slopes and, hence, conditions. Conventional two-way ANOVA procedures were not possible because of the unbalanced nature of the tissue.
EXERCISE AND SR Ca\textsuperscript{2+} CYCLING IN MUSCLE

Table 1. Respiratory gas-exchange measurements during progressive exercise in normoxia and hypoxia

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre</th>
<th>137</th>
<th>214</th>
<th>250</th>
<th>300</th>
</tr>
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<tbody>
<tr>
<td>(V_{O_2}), l/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.42±0.02</td>
<td>1.86±0.08</td>
<td>2.98±0.12</td>
<td>3.23±0.16</td>
<td>4.07±0.15</td>
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<tr>
<td>H</td>
<td>0.39±0.02</td>
<td>1.86±0.11</td>
<td>2.83±0.13</td>
<td>3.21±0.11</td>
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<tr>
<td>(V_{CO_2}), l/min</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>N</td>
<td>0.38±0.02</td>
<td>1.75±0.11*</td>
<td>3.17±0.09*†</td>
<td>3.20±0.16††</td>
<td>5.03±0.18</td>
</tr>
<tr>
<td>H</td>
<td>0.36±0.03</td>
<td>1.94±0.11*</td>
<td>3.20±0.16††</td>
<td>3.98±0.15††</td>
<td></td>
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<tr>
<td>(V_{ETRPS}), l/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N</td>
<td>11.3±0.77</td>
<td>35.4±1.2* †</td>
<td>61.8±2.6* †</td>
<td>79.7±4.5† †</td>
<td>116±4.0</td>
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<tr>
<td>H</td>
<td>12.2±0.56</td>
<td>42.2±2.3* †</td>
<td>79.3±4.5† †</td>
<td>104±4.3† †</td>
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<td>N</td>
<td>0.88±0.02</td>
<td>0.92±0.02</td>
<td>1.03±0.01</td>
<td>1.13±0.01</td>
<td>1.20±0.02</td>
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<tr>
<td>H</td>
<td>0.96±0.01</td>
<td>0.98±0.01</td>
<td>1.09±0.02</td>
<td>1.21±0.02</td>
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</table>

Values are means ± SE (n = 9). Pre, preexercise; PO, power output [note: 250 and 300 represent the PO at fatigue in hypoxia (H) and normoxia (N), respectively]; \(V_{O_2}\), oxygen consumption; \(V_{CO_2}\), carbon dioxide production; \(V_{ETRPS}\), expiratory volume; RER, respiratory exchange ratio. Main effects for condition (\(P < 0.05\)) were found for \(V_{CO_2}\), \(V_{ETRPS}\), and RER. For \(V_{ETRPS}\), RER and \(V_{CO_2}\), H > N. Main effects (\(P < 0.05\)) for time were found for \(V_{O_2}\) and RER. For \(V_{O_2}\) and RER, Pre < 137 < 214 < 250 W. *Significantly different from Pre (\(P < 0.05\)). †Significantly different from 137 W (\(P < 0.05\)). ††Significantly different from 214 W (\(P < 0.05\)).

Table 2. Effects of progressive exercise in normoxia and hypoxia on kinetic characteristics of sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase activity in vastus lateralis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre</th>
<th>1.86</th>
<th>2.98</th>
<th>3.21</th>
<th>4.07</th>
</tr>
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<tbody>
<tr>
<td>(V_{max}), (\mu)mol min(^{-1}) g protein(^{-1})</td>
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<td></td>
<td></td>
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<tr>
<td>N</td>
<td>161±11</td>
<td>147±8.5</td>
<td>158±9.4</td>
<td>152±11</td>
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</tr>
<tr>
<td>H</td>
<td>167±11</td>
<td>164±13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n_H)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1.7±0.1</td>
<td>2.0±0.2</td>
<td>2.0±0.2</td>
<td></td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>H</td>
<td>2.0±0.2</td>
<td>1.8±0.2</td>
<td></td>
<td></td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>(C_{\text{aho}}), nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>744±133</td>
<td>889±209</td>
<td>832±185</td>
<td></td>
<td>791±108</td>
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<td>869±133</td>
<td>886±95</td>
<td></td>
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<td>838±84</td>
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</table>

Values are means ± SE (n = 9). Pre, preexercise. \(V_{O_2}\) corresponds to average \(V_{O_2}\) when biopsies were performed. \(V_{max}\), maximal Ca\textsuperscript{2+}-ATPase activity; \(n_H\), Hill coefficient, determined from Hill plots by using the relationship between Ca\textsuperscript{2+} concentration and Ca\textsuperscript{2+}-ATPase activity and the section of the curve that corresponded to 20–80% of the maximal Ca\textsuperscript{2+}-ATPase activity; \(C_{\text{aho}}\), Ca\textsuperscript{2+} concentration at half-maximal Ca\textsuperscript{2+}-ATPase activity.

J Appl Physiol • VOL 97 • JULY 2004 • www.jap.org
Progressive exercise resulted in a reduction in Ca\textsuperscript{2+}/ATPase activity in normoxia that was independent of [Ca\textsuperscript{2+}] but only at fatigue (Fig. 3). Reductions in Ca\textsuperscript{2+} uptake also occurred during hypoxia. No differences were observed in Ca\textsuperscript{2+} uptake between normoxia and hypoxia as determined by the slopes of the regression lines. Coupling ratios, defined as the ratio between Ca\textsuperscript{2+} uptake and Ca\textsuperscript{2+}-ATPase activity, were unaffected either during exercise or between conditions (Fig. 4).

The effects of exercise and condition on Ca\textsuperscript{2+} release were determined by analyzing two phases of Ca\textsuperscript{2+} release (phase 1 and phase 2; Fig. 5). Ca\textsuperscript{2+} release was depressed during exercise for each condition in an intensity-dependent manner but only for phase 2 (Fig. 6). For normoxia, before exercise was greater than fatigue. For hypoxia, before exercise was greater than fatigue. No differences in slopes were observed between normoxia and hypoxia for either phase.

**DISCUSSION**

In this study, we have investigated the hypothesis that, in normoxia, progressive disturbances in SR function would oc-

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**Fig. 2.** Representative curves for a single participant illustrating the Ca\textsuperscript{2+}-dependent changes in Ca\textsuperscript{2+}-ATPase activity in normoxia and hypoxia. These measurements were made at rest. The kinetic properties of the Ca\textsuperscript{2+}-ATPase were obtained from this curve.

**Fig. 3.** Ca\textsuperscript{2+}-dependent changes in Ca\textsuperscript{2+} uptake during progressive exercise in normoxia and hypoxia. Ca\textsuperscript{2+} uptake was assessed at 4 different systolic free Ca\textsuperscript{2+} concentrations ([Ca\textsuperscript{2+}]\textsubscript{f}; 500, 1,000, 1,500, and 2,000 nM). V\textsubscript{O2}, O\textsubscript{2} consumption assessed during the progressive exercise tests. Main effects (P < 0.05) for [Ca\textsuperscript{2+}]\textsubscript{f} and exercise were observed. For Ca\textsuperscript{2+}, 2,000 > 1,500 > 1,000 > 500 nM. For exercise, before exercise was greater than fatigue. Values are means ± SE (n = 9).

**Fig. 4.** Coupling ratios obtained during progressive exercise in normoxia and hypoxia. Coupling ratio is defined as the ratio of Ca\textsuperscript{2+} uptake (2,000 nM) to Ca\textsuperscript{2+}-ATPase activity. Values are means ± SE (n = 10) determined at rest and during exercise to fatigue.

**Fig. 5.** Representative trace for the measurement of Ca\textsuperscript{2+} uptake and Ca\textsuperscript{2+} release. Ca\textsuperscript{2+} uptake was initiated with the addition of ATP to the homogenate. After Ca\textsuperscript{2+} levels had plateaued (baseline), 4-chloro-m-cresol (4-CMC) was added to the cuvette to initiate Ca\textsuperscript{2+} release. Uptake, tracing for Ca\textsuperscript{2+} uptake by sarcoplasmic reticulum. Two phases of Ca\textsuperscript{2+} release were identified: a fast phase (phase 1) and a slow phase (phase 2).
Fig. 6. Changes in Ca\textsuperscript{2+} release during progressive exercise in normoxia and hypoxia during phase 1 (A) and phase 2 (B). Ca\textsuperscript{2+} release has been plotted against \(\dot{V}O_2\) before exercise and during progressive exercise. Reductions (\(P < 0.05\)) in phase 2 of Ca\textsuperscript{2+} release were observed during exercise for each condition. *Significantly different from before exercise (\(P < 0.05\)). †Significantly different from 70% \(\dot{V}O_2\) peak in normoxia (\(P < 0.05\)). ‡Significantly different from 50% \(\dot{V}O_2\) peak in normoxia (\(P < 0.05\)).

In this study, we have used 4-CMC as the CRC-releasing agent. The selection of 4-CMC, instead of AgNO\textsubscript{3}, which has previously been commonly employed as a releasing agent (9), was based on a recent publication by our group (47). In that study, our laboratory found that AgNO\textsubscript{3}, in contrast to 4-CMC,
induces Ca\(^{2+}\) release by acting on both the CRC and Ca\(^{2+}\)-ATPase. We have also been able to identify two phases of Ca\(^{2+}\) release, phase 1 and phase 2. We have observed that only reductions in phase 2 were observed with exercise. At present, the physiological significance of this observation is not clear because, in our in vitro system, the Ca\(^{2+}\)-release rates occur at a much slower rate than observed in single-fiber and isolated-channel preparations (9). Our homogenate system is based on a preparation from whole muscle and consequently contains all of the proteins regulating SR behavior. This factor would appear important in the regulation of Ca\(^{2+}\) release and explain, at least in part, differences that exist between the homogenate system and other preparations, such as isolated vesicles and single channels. It must be acknowledged, however, that, regardless of the preparation, some functional alterations could be expected during the homogenization process.

The reduction in Ca\(^{2+}\) release that we have observed with our progressive exercise protocol probably results from a decrease in the number of functional CRC (9). Although definitive evidence is lacking, it is strongly suspected that oxidative and/or nitrosylative processes mediated by ROS are involved in the structural modifications to the CRC that occur (9, 44). It would be expected that ROS would accumulate during progressive exercise given the increase in OxPhos and the increase in inosine 5’-monophosphate (IMP) that occurs (16). It has been estimated that 2–4% of OxPhos results in ROS generation by the electron transport system (39, 41). In addition, the conversion of IMP to hypoxanthine and xanthine by xanthine oxidase also increases ROS formation (39, 41). Conceivably, the increase in ROS generation by xanthine oxidase during exercise would occur given the larger IMP accumulation (35). It is possible that the increase in ROS was insufficient to result in a greater reduction in Ca\(^{2+}\) release with hypoxia compared with normoxia. At present, it is not clear why reductions in Ca\(^{2+}\)-ATPase activities during progressive exercise did not occur in either normoxia or hypoxia, given the apparent sensitivity of this enzyme to ROS (23).

A potential factor that could influence our measurements of the SR properties has to do with potential oxidation during the homogenate preparation. In our study, as well as most similar type studies, the DTT, a sulfhydryl-reducing agent, was not added. Consequently, it would be expected that some oxidation would occur, possibly masking the actual changes that occurred with exercise. DTT was not added because of the possibility that the effects of exercise, if due to oxidation, would be reversed.

In this study, we have confirmed what has been reported on many previous occasions, namely that, with hypoxia (inspired O\(_2\) fraction = 0.14), VO\(_2\) peak is reduced compared with normoxia (15, 33). The ~23% reduction that was observed in VO\(_2\) peak is consistent with what has been reported earlier in untrained subjects under similar conditions (20). We have also been able to demonstrate, again similar to previous studies (20), that, over a range of submaximal POs, compensatory adjustments allow OxPhos to remain similar or nearly similar to normoxia despite a lower arterial O\(_2\) saturation and arterial O\(_2\) content. Even at the highest PO, attained at fatigue in hypoxia, we could find no differences from normoxia when matched to the same PO. Some insight into these adjustments can be obtained from the minute ventilation and respiratory exchange ratio measurements, both of which were increased in hypoxia compared with normoxia at similar POs. Increased ventilation in conjunction with increased blood flow to working muscles appears important in minimizing the reductions in arterial O\(_2\) delivery in hypoxia (50, 51). Our results indicate that a substantial blunting of PO and VO\(_2\) peak can occur even though OxPhos is relatively well protected throughout submaximal exercise. This observation could suggest that fatigue may occur somewhat independently of a failure in ATP supply by OxPhos. Conceivably, fatigue could also prevent further increases in OxPhos (and VO\(_2\) peak) even though delivery, extraction, and utilization of O\(_2\) by mitochondria have not been fully exploited. Failure in E-C coupling as a cause of fatigue remains an inviting possibility in this regard.

To examine differences between normoxic and hypoxic conditions in Ca\(^{2+}\)-cycling properties, it was not possible to use conventional two-way ANOVA procedures for repeated measures as a result of the unbalanced nature of our tissue-sampling schedule. Although the first exercise samples occurred at the same VO\(_2\) (and PO), this was not possible at the higher exercise intensities due to the randomization of conditions and ethical limitations in the number of biopsies that could be performed. To examine for differences between conditions, we have used regression analyses and slopes from the lines of best fit for the relationship between VO\(_2\) (or PO) and the property of interest. These plots clearly demonstrate a trend (\(P > 0.05\)) toward a greater effect of hypoxia on Ca\(^{2+}\) uptake and Ca\(^{2+}\) release. It should be emphasized that this approach using regression analyses depends on the assumption of linearity between the measured parameters and PO.

Although not measured in this study, reductions in the [Ca\(^{2+}\)]\(_t\) time integral remain a viable possibility to explain the inability to continue the progressive exercise task in both normoxia and hypoxia. Decreases in [Ca\(^{2+}\)]\(_t\) time integral could occur specifically as a consequence of decreases in Ca\(^{2+}\) release. Reductions in the Ca\(^{2+}\) time integral, mediated by decreases in Ca\(^{2+}\) release, have been previously shown to associate with fatigue in repetitively stimulated mouse muscle fibers (2). The fact that the depression in Ca\(^{2+}\) release was comparable to normoxia in hypoxia at fatigue, even though PO was considerably lower in hypoxia, is also suggestive that impaired Ca\(^{2+}\) cycling may be involved in the early fatigue observed during progressive exercise in this condition.

It must be emphasized that other, more central processes may be involved in the inability to continue progressive exercise. Because our measurements were conducted on homogenates under supposedly optimal in vitro conditions, the depression in Ca\(^{2+}\) release can be attributed directly to disturbances in the CRC. However, in vivo, other processes such as an inability of the sarcolemma and T tubule to conduct a repetitive action potential and/or a deficit in the mechanical signaling between the T tubule and the CRC could lead to reductions in Ca\(^{2+}\) release (2). It should also be emphasized that with progressive exercise profound changes occur in the intracellular environment, which may impair Ca\(^{2+}\) cycling as well as a variety of other excitation and contraction processes (53). Consequently, isolating the direct site and process involved in fatigue during voluntary activity is not possible.

In summary, our results demonstrate that, during progressive exercise to fatigue in normoxia, disturbances in SR Ca\(^{2+}\) cycling occur as indicated by the in vitro measurements of Ca\(^{2+}\) uptake and Ca\(^{2+}\) release. With hypoxia, similar distur-
bances in Ca\(^{2+}\) cycling occur but at a reduced PO\(_{\text{peak}}\) and VO\(_{\text{2peak}}\). These findings raise the possibility that fatigue, both in normoxia and hypoxia with progressive exercise, is related to an inability to regulate the [Ca\(^{2+}\)]\(_i\) integral.

**GRANTS**

Financial assistance for the study was provided by grants to H. Green from the National Sciences and Engineering Research Council of Canada.

**REFERENCES**


